Department of Global Resources

The mission of the Department of Global Resources is expressed by 2 broad global environmental research goals: to assess the agro-ecological impacts of unusual climatic variations and global warming, and to develop adaptive technical and policy measures to reduce any adverse impacts by clarifying climate-change mechanisms and by monitoring and modeling. These missions are the concern of 2 research groups— the Agro-Meteorology Group and the Ecosystems Group— and 3 teams— the Greenhouse Gas Emission Team, the Food Production Prediction Team, and the Ecosystem Gas Exchange Team.

Research projects are initiated in domains such as 1) prediction of food production under global environmental variability; 2) elucidation of the impacts of global climate change on agro-ecosystems; 3) estimation of greenhouse gas emissions from agricultural activities and development of measures that can be used to minimize emissions; 4) determination of the effects of human activity, especially the flow of carbon and nitrogen; 5) development of techniques for remote sensing and multivariate statistical analysis; and 6) assessment of changes in rural land use.

Some of our research projects are involving in the Global Warming Research Initiative and Water Resources Research Initiative, which are managed under the Council for Science and Technology Policy. The goal of our projects is to formulate an adaptable scenario of greenhouse gas emissions that will help stabilize climate change within a range that is compatible with human civilization.

1) Agro-Meteorology Group

The mission of the Agro-Meteorology Group is to clarify predictions of the impacts on agricultural ecosystems of climate change and elevated atmospheric CO_2 levels and of the resulting atmospheric changes in such ecosystems. The Agro-Meteorology Group consists of 3 units: 1) Climate Resources Unit; 2) Atmospheric Impacts Unit; and 3) Air Quality Conservation Unit.

The research aim of the **Climate Resources Unit** is to develop monitoring techniques for evaluating the effects of climate change and elevated CO_2 on agricultural water resources, and to develop methods for predicting these changes. The research objective of the **Atmospheric Impacts Unit** is to develop models for predicting the effects of elevated atmospheric CO_2 on agricultural ecosystems by analysis of the results of free air CO_2 enrichment (FACE) experiments. The research of the **Air Quality Conservation Unit** is focused on clarifying the processes of emission, diffusion, and deposition of air components such as trace gases, pollens, and dust in agricultural ecosystems.

In FY 2004, the following research was conducted by the 3 units: 1) investigation and prediction of spatio-temporal change in agricultural water resources; 2) development of a dynamic water model for evaluating agricultural water quality on a regional scale; 3) impact assessment of climate change from the viewpoint of agricultural production management; 4) modeling of spatial and temporal dynamics of the soil carbon budget; 5) prediction of the impacts of atmospheric CO_2 increase on crop production and water use; 6) process-based modeling of agricultural ecosystems under rising temperatures and increasing atmospheric CO_2 concentrations; 7) investigation of the impact of increasing atmospheric CO_2 on heat stress in crop plants; 8) modeling and simulation of canopy microclimate and fluid dynamics for developing open-air warming systems; 8) determination of the developmental and morphological responses of rice to elevated atmospheric CO_2 concentrations; 9) development of a system for estimating and testing cultivar coefficients of rice development models utilizing existing data; 10) modeling and estimation of the emission and diffusion processes controlling air quality in agro-ecosystems; 10) examination of the relationship between ground surface conditions and aeolian dust events; 11) assessment of the effects of temporal and spatial variations in the bio-meteorological environment on alpine grassland ecosystems; 12) assessment of the climate mitigation function of agricultural land; and 13) a feasibility study on the linkage of regional climate change with intra-seasonal and inter-annual variations in the concentrations of ozone and its precursors over Japan.

Twenty-two original research papers were published in international and domestic journals in FY 2004. The 21st Meteorology Workshop, entitled “Aeolian dust (kosa) problem and agricultural activity”, was organized by the Air Quality Conservation Unit and held at NIAES on 3 March 2005. Dr. Mingyuan Du, a senior researcher in the Air Quality Conservation Unit, won the progress award of the Japanese Association for Arid Land Studies for his outstanding research on climate and human activity in the arid areas of China, with a particular focus on the Taklamakan Desert.

Topic 1: CO_2, CH_4, and N_2O fluxes from soybean and barley double-cropping in relation to tillage in Japan

Human activity is leading to the atmosphere emission

of vast quantities of greenhouse gases and is thus making global warming a reality. If we are to prevent global warming, we will need to control the emission of these greenhouse gases. In terms of carbon equivalents (the sum of emissions of each type of greenhouse gas multiplied by the gas's global warming potential (using factors of 1 for CO_2, 21 for CH_4 and 310 for N_2O)), the total amount of greenhouse gases emitted by the agricultural sector as a proportion of that from all sectors in Japan in FY 2001 was 2.6%; thus, the contribution of the agricultural sector is low. However, the agricultural sector emits high proportions of the total amount of CH_4 (67%; enteric fermentation 33% and rice cultivation 29%) and the total amount of N_2O (57%; manure management 34% and agricultural soil 23%) (National GHGs Inventory Report of Japan 2003, Ministry of the Environment of Japan, 2003). Therefore, the agriculture sector needs to reduce its emissions of CH_4 and N_2O.

Upland fields emit large volumes of N_2O and absorb small amounts of CH_4. Agricultural soils also emit CO_2, and soil CO_2 emissions are strongly dependent on plant and soil microbial activity, which in turn are influenced by temperature. N_2O and CO_2 emissions from upland soils are responsive to management, including appropriate selection of crop species, tillage, and application of fertilizers and manure. CO_2 emission due to soil respiration is often stimulated by tillage. On the other hand, no-tillage cultivation may increase N_2O emissions because of the likelihood of the soil being maintained in a wet condition and becoming anaerobic. Although the number of studies of greenhouse gas emissions from agricultural fields is increasing, there is still only limited research into the reduction of emissions by improvement of farming management.

No-tillage cultivation (Photo 1) is of interest as a farming management technique for greenhouse gas emission reduction. Tillage cultivation accelerates carbon oxidation of organic residue in soil by increasing soil aeration, and it accelerates soil erosion by increasing exposure to wind and rain. In contrast, no-tillage cultivation can reduce water and wind erosion, conserve soil moisture, and reduce fuel costs. About 37% of the land farmed in the United States is now managed by the use of conservational tillage systems, including no-tillage. However, because Japan has a mild and humid climate that allows thick weed growth, plowing (conventional tillage) is yet usually conducted as a control technique for weed.

To examine whether no-tillage cultivation of upland fields mitigates greenhouse gas emissions from agriculture, we measured seasonal changes in CO_2, CH_4, and N_2O emissions under conventional tillage and no-tillage

Photo 1 Soil respiration chamber placed among soybeans under no-tillage cultivation.

over a whole year (13 May 2002 to 13 May 2003) at Tsukuba, Japan, which has a temperate climate. The soil respiration rate increased with increasing soil temperature (Fig. 1). The annual soil respiration rate was 2845 ± 967 g CO_2 m^{-2} y^{-1} in the conventional tillage plot and 2198 ± 656 g CO_2 m^{-2} y^{-1} in the no-tillage plot; the annual soil respiration rate under no-tillage conditions thus showed a 23% decrease compared with that under conventional tillage, but the difference was not significant. When organic matter was incorporated into the soil of the conventional tillage plot by plowing in the crop residue after harvest, the soil respiration rate and N_2O flux increased rapidly (Fig. 2). The majority of the difference between the conventional tillage and no-tillage plots in terms of annual soil respiration rate (647 g CO_2 m^{-2} y^{-1}) was accounted for by the difference in the rate during

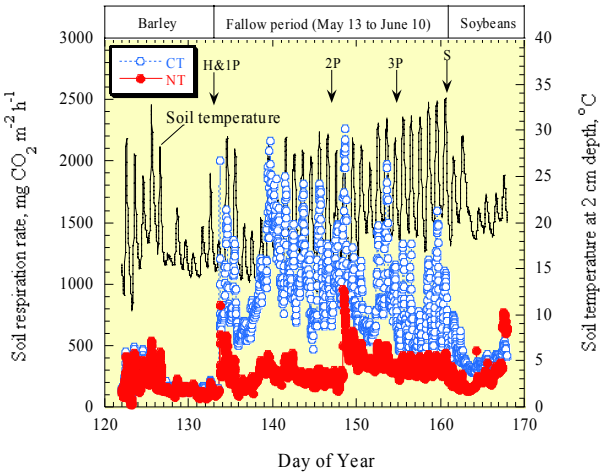

Fig. 1 Daily changes in soil respiration rate over 50 days (2 May to 16 June 2002) during barley growing, harvesting (H), and 3 plowings (1P to 3P) and during sowing (S) of soybeans, with data on soil temperature at a depth of 2 cm. CT: conventional tillage; NT: no-tillage; FA: fertilizer application.

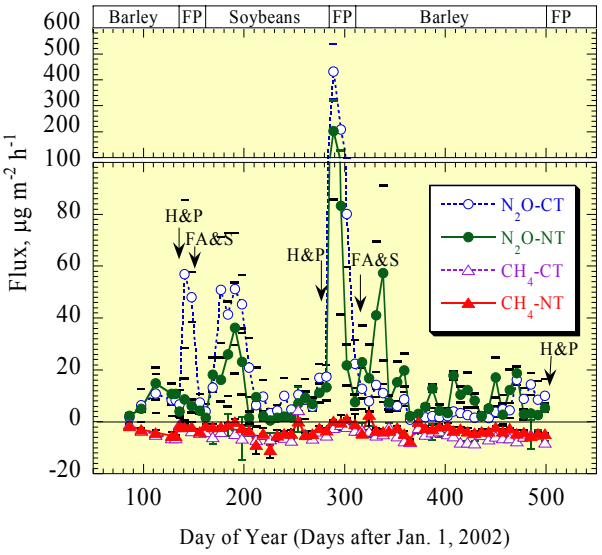

Fig. 2 Seasonal changes in CH_4 and N_2O fluxes over the whole year from conventional tillage and no-tillage plots under a double-cropping agro-ecosystem. CT: conventional tillage; NT: no-tillage; H: harvesting; P: plowing; FA: fertilizer application; S: sowing. The horizontal bars above each symbol represent standard deviations.

fallow periods (444 g CO_2 m^{-2} per period). Comparison of total greenhouse gas emissions in terms of carbon equivalents from soybean and barley double-cropping using conventional tillage and no-tillage showed that a reduction in carbon emission by 183 g carbon m^{-2} y^{-1} was possibly by the use of no-tillage cultivation. These results clearly show that no-tillage cultivation is one of the most promising strategies for the mitigation of greenhouse gas emissions by the agricultural sector. (I. Nouchi)

Topic 2: Rice yield enhancement by CO_2 elevation is negatively correlated with acceleration of heading: results from 5 years of chamber studies

Varying degrees of rice (*Oryza sativa* L.) yield enhancement by elevation of CO_2 concentration ($[CO_2]$) have often been reported. To identify the reasons behind this variation in yield enhancement by CO_2 elevation, we analyzed the results of experiments conducted in 6 naturally sunlit controlled-environment chambers over 5 years (1998–2002). Rice plants (cv. Nipponbare) were grown season-long under ambient (354–383/397–448 μmol mol^{-1}; day/night) and elevated (670–721/702–780 μmol mol^{-1}) $[CO_2]$, using 3 chambers for each experiment. Air temperatures inside the chambers were controlled at outside levels. Relative humidity was kept at 77% to 80%. Total nitrogen application was 8 g m^{-2} in 1998 and 1999 and 12 g m^{-2} in 2000–2002. In 2001 we added to each chamber 2 subplots in which the timing of topdressing was different.

Leaf area index (LAI) at the heading stage was largely influenced by total N applied, but the effect of elevated $[CO_2]$ was not significant, although LAI was reduced by elevated $[CO_2]$ in 3 of the 5 years. Final total dry weight was significantly increased (by 8.0% to 18.7%) by elevated $[CO_2]$ in all 5 years. Enhancement of final total dry weight by elevated $[CO_2]$ at each N level was similar across years. Interestingly, the enhancement ratio was not altered when the timing of topdressing was changed with the same total N application in 2001, although topdressing at the heading stage increased total dry weight more than at the panicle initiation stage under both $[CO_2]$ treatments. Days to heading were significantly shortened by elevated $[CO_2]$ by 2.6 to 8.0 days. Grain yield was significantly increased by elevated $[CO_2]$ to varying degrees, ranging from 4.1% to 22.4%, but the relationship between grain yield and final total dry weight enhancement was not clear. In contrast, a strong negative relationship was found between grain yield enhancement and reduction in number of days to heading under elevated $[CO_2]$ (Fig. 3). These results suggest that the degree of acceleration of plant development can have a marked impact on rice yield responsiveness to elevated $[CO_2]$. (H. Sakai)

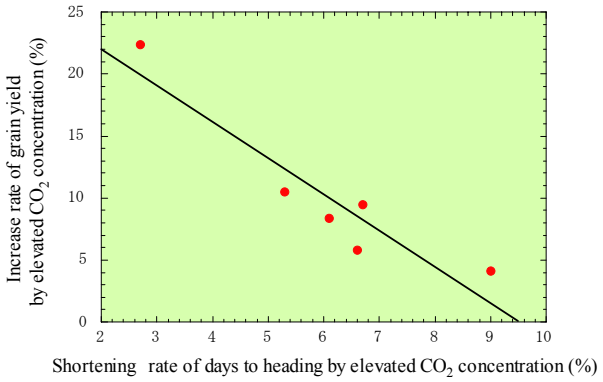

Fig. 3 Relationship between change in rate (%) of grain yield and change in rate of reduction (%) in number of days to heading under elevated CO_2 concentration over 5 years (1998–2002).

2) Ecosystems Group

The Ecosystems Group consists of five units and carries out both practical and fundamental research. The **Agro-Ecological Sensing Unit** is developing remote sensing and modeling methods for monitoring plant and environmental dynamics in agricultural and natural ecosystems on the basis of optical and electromagnetic

measurements ranging from leaf scale to regional scale. The **Statistics Unit** is developing novel statistical methodologies for sampling, classifying, and analyzing agro-environmental data. The **Material Ecocycling Unit** is studying nitrogen and nutrient flows in agro-ecosystems to evaluate the relationship between anthropogenic activities and material cycles in Japan and East Asia. The **Ecological Management Unit** is studying historical changes in the spatial structure of rural ecosystems and the conservation and management of the wildlife that inhabits environments of the Kanto District. The **Remote Sensing Unit** is determining the environmental characteristics that can be observed at a regional scale through satellite imaging systems such as multi-band and multi-polarization SAR (Synthetic Aperture Radar) and MODIS (Moderate Resolution Imaging Spectroradiometer) (see Topic). In FY 2004, we conducted 12 research projects funded by MAFF (Ministry of Agriculture, Forestry and Fisheries) and the Ministry of the Environment. Our researchers attended 9 international meetings related to anthropology, ecology, remote sensing, and statistics. The researchers also made 10 overseas trips—to Canada, China, Laos, Vietnam, France, and Thailand—for fieldwork, cooperative projects, and research exchange. Domestically, we made a total of 36 presentations at academic meetings on statistics, remote sensing, geography, environmental sciences, and anthropology.

***Topic:* An accurate technique for detecting planted rice paddy fields using spaceborne radar and geographic information system (GIS)**

Every year the Japanese Government collects about 40 000 nationwide survey samples to determine the distribution of planted rice paddies. Because this requires a massive amount of fieldwork, the government has been looking for a low-cost and fast alternative. Remote sensing techniques, and especially spaceborne radar techniques, fit for this request well.

Synthetic aperture radar (SAR) is a kind of imaging radar that images the strength of radar waves backscattered from the ground surface. As the radar microwave has a longer wavelength than visible light and can penetrate cloud, SAR can acquire cloud-free images independent of weather conditions. This ability is vital for the use of SAR in gathering data on rice.

In SAR imagery, the surface of open water appears dark, because most of the radar wave is reflected, as by a mirror, and only a little is scattered back to the sensor. On the other hand, vegetated surfaces appear bright, because leaves and stems scatter radar waves more isotropically, and stronger scatter returns to the sensor (Fig. 1, top).

On the basis of these features, it is possible to detect planted rice paddies by comparing the SAR images acquired in the rice transplanting and growing seasons. In accordance with the government policy of reducing the rice acreage, some paddy fields are kept unplanted but inundated for weed control. Such fields are distinguishable from planted fields (Fig. 1).

However, the detection performance of SAR drops for paddy fields located on mountainous region because the image position is often not correct. It is caused by the principle of SAR. To reduce misclassification, such areas, which are considered unusable for rice cultivation, are masked out of the satellite-derived distribution. The mask covering these areas is created from 1:25 000 digital maps by image processing and GIS manipulation. Because these digital maps are available for the whole territory of Japan, we can create a mask for any region of interest in a uniform manner (Fig. 2).

The distribution of the planted area on the Saga Plain

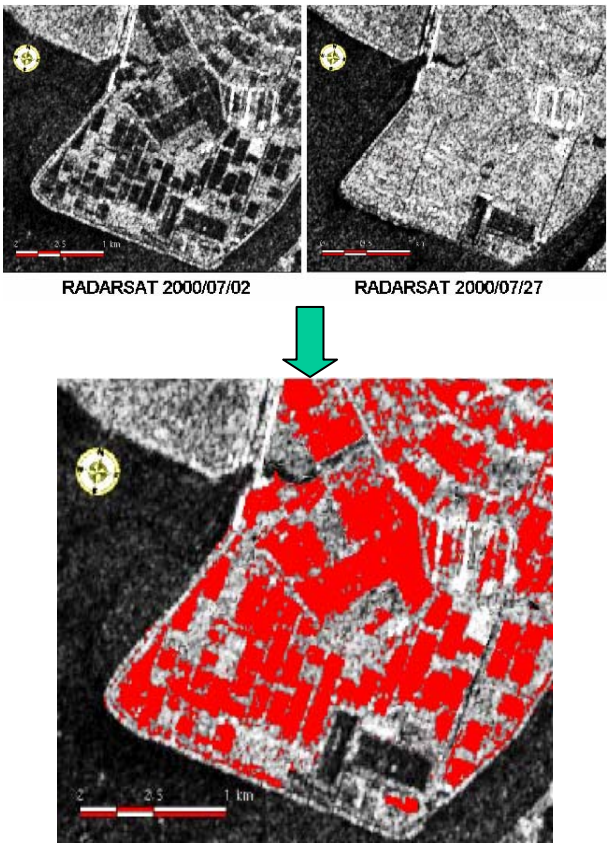

Fig. 1 Detection of planted rice paddy fields on the Saga Plain using SAR images. Specular reflection from water in the transplanting season shows up as dark areas, and large backscatter values from rice vegetation in the growing season appear bright. Unplanted rice fields kept inundated for weed control are found to the lower right of the images.

was determined by using RADARSAT C-band (wavelength 6 cm) images acquired on 2 July and 27 July 2000, and the total area was compared with that determined by the conventional statistical survey procedure. The total area determined by using the SAR technique was 102% of that obtained by the conventional procedure. The RMSE of the area of 26 municipalities on the plain was 85 ha (Fig. 3). A comparison of the same area in 2001 gave equivalent results of 101.5% and 54 ha.

The accuracy and stability of this technique are therefore sufficient for it to be used as an alternative to the procedure currently used to determine the area of paddies planted to rice in Japan. (N. Ishitsuka, H. Ohno and T. Sakamoto)

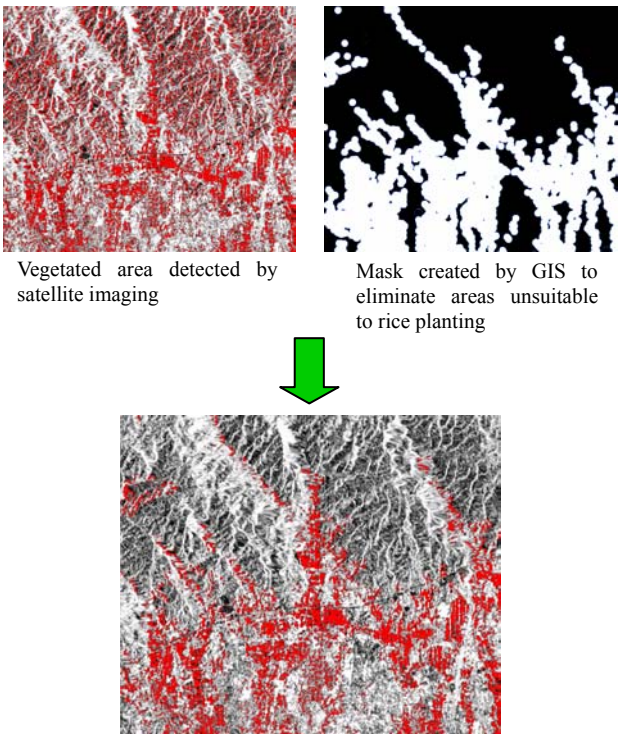

Fig. 2 Mask processing of GIS data. To reduce misclassification, a mask covering areas where rice is never cultivated is applied to detect planted rice paddy fields on satellite images.

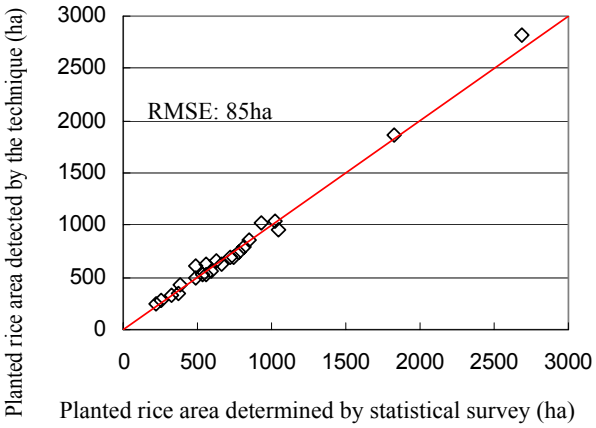

Fig. 3 Comparison of the area of rice paddies planted on the Saga Plain in 2000, as determined by the SAR technique and by the current statistical technique.

3) Greenhouse Gas Emission Team

Considerable attention has been paid in recent years to the likelihood of significant changes in world climate owing to the presence of increased atmospheric concentrations of greenhouse gases (GHGs). GHGs, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), can absorb thermal radiation from the surface of the earth and thus contribute to the warming of the atmosphere. The Intergovernmental Panel on Climate Change (IPCC) has reported that concentrations of atmospheric GHGs and their radiative forcing have continued to increase as a result of various human activities.

Agriculture contributes to over 20% of global anthropogenic GHG emissions. In particular, 55% to 60% and 65% to 80% of total emissions of CH_4 and N_2O, respectively, are derived from agricultural sources. These GHGs are emitted to the atmosphere as a result of accelerated turnover of carbon and nitrogen in agricultural soils and the surrounding environment through increased input of chemical and organic fertilizers and other agro-materials. This increased input also results in increased emission of nitrogen oxide (NO) and ammonia (NH_3), which are precursors of acid rain, and in pollution of rivers and groundwater by leaching of nitrogen and carbon components. Land-use change and burning of plant biomass increase emissions of CO_2, CH_4, N_2O, and other trace gases such as carbon monoxide (CO), hydrogen (H_2), and halocarbons.

The Greenhouse Gas Emission Team studies emission and absorption of these environmentally important gases in association with different land uses and agricultural management. The activities of the team are based on field measurements of GHG exchange, laboratory experiments, data interpretation, and modeling. The goals of the team are 1) to quantify and model the processes of GHG emission and absorption (mechanism); 2) to estimate the rates of GHG emission and absorption (inventory); and 3) to develop promising and feasible technologies that reduce GHG emissions (mitigation) (Fig. 1). The studies have been developed to address scientifically and socially important questions related to the environmental impacts of agriculture.

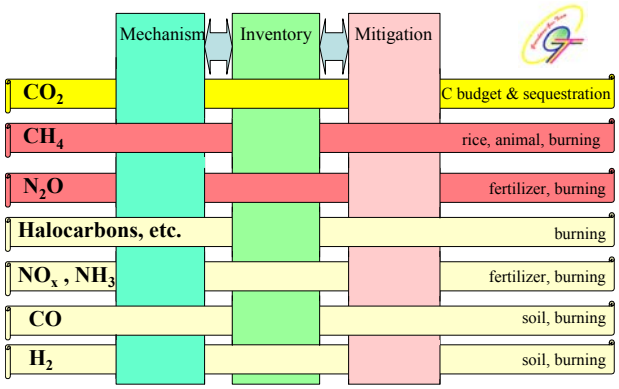

Fig. 1 Research targets of the Greenhouse Gas Team.

Photo Overview of furnace used for biomass burning experiments.

***Topic:* Laboratory combustion experiment for estimation of greenhouse gas emissions from biomass burning**

Biomass burning is known as one of the major sources of emission of greenhouse gases (e.g. CH_4, N_2O, CO_2) from agro-ecosystems. The emission factors of greenhouse gases from biomass combustion have large uncertainties, as they are controlled by several kinds of combustion parameters such as water content, density and porosity of combusted materials, environmental humidity, and fire temperature. Using a closed-shell type combustion furnace (see photo), we simulated the factors controlling greenhouse gas emissions from several types of biomass combustion.

To make a closed-shell combustion system in which the inside air pressure and humidity were controllable and the total volume of gas flow was measurable, we customized a commercially available furnace. The furnace was equipped with an electric igniter with nichrome wire and stainless steel ports for supplying fresh air and extracting smoke gases. An electronic balance was installed inside the furnace and connected to a PC to measure the transition of biomass weight during combustion. Atmospheric pressure was maintained inside the furnace by using a 5-L pressure-compensating plastic bag. Fresh air (synthesized by mixing pure O_2 and N_2) from gas cylinders was supplied at flow rate (0.5 to 1.0 L/min). A gas-tight syringe for use as a water-injection port was added to the fresh-air supply line to adjust the humidity.

Various parameters of biomass sample and combustion conditions are shown in Table 1, together with

Table 1 Parameters of biomass samples and combustion conditions, and measured emission factors of CO_2, CO, and CH_4.

Biomass sample	Water Content (g/g)	Humidity (%)	Porosity (cm^3/cm^3)	Density (g/cm^3)	MCE	Fire Temp. (°C)	Weight (g dm)	Carbon content (g)	†Ef-CO_2 (x10)	‡Ef-CO ($x10^2$)	¶Ef-CH_4 ($x10^4$)	*GCR (gC/gC)
Abies sachalinensis	0.00	0.20	0.11	0.98	0.973	852	3.20	1.58	9.05	1.57	5.34	0.514
Abies sachalinensis	0.30	0.20	0.11	0.98	0.984	897	2.23	1.10	14.96	1.57	3.97	1.094
Abies sachalinensis	0.30	0.60	0.11	0.98	0.985	901	2.46	1.22	10.32	1.02	2.83	0.750
Acer pictum	0.00	0.20	0.48	1.66	0.972	847	2.70	1.27	4.30	0.78	3.79	0.257
Betula ermanii	0.00	0.20	0.49	1.80	0.973	850	3.30	1.65	2.65	0.64	3.54	0.151
Betula ermanii	0.30	0.20	0.49	1.80	0.987	913	2.92	1.46	4.02	0.33	0.79	0.289
Betula ermanii	0.30	0.60	0.49	1.80	0.984	897	3.08	1.54	10.15	1.06	2.54	0.731
Kalopanax pictus	0.00	0.20	0.54	1.86	0.965	817	3.00	1.35	10.75	2.44	9.25	0.676
Kalopanax pictus	0.30	0.20	0.54	1.86	0.978	872	2.23	1.01	1.31	0.19	0.36	0.105
Kalopanax pictus	0.30	0.60	0.54	1.86	0.977	867	2.31	1.04	15.40	2.31	11.32	1.243
Picea glehnii Mast.	0.00	0.20	0.37	1.51	0.977	869	4.10	1.89	7.36	1.09	2.46	0.446

†Ef-CO_2: emission factor for CO_2
‡Ef-CO: emission factor for CO
¶Ef-CH_4: emission factor for CH_4
*GCR: Gas Conversion Ratio

measured emission factors for CO_2, CO, and CH_4. There was a negative correlation between sample density and porosity, with a slope value of 2.03 ($R^2 > 0.99$), indicating that heavier wood materials in this experimental series had lower porosities. The dry weight of each sample was measured after keeping the sample in a desiccator with silica gel for 1 week.

Emission factors were calculated by dividing the weight of the emitted gas by the dry weight of the biomass sample. The gas conversion ratio (GCR) was calculated by dividing the total weight of CO_2, CO, and CH_4 by the dry weight of the biomass sample. Modified combustion efficiency (MCE), as defined in Eq. 1 (Yokelson et al., 1997), was used to estimate the average combustion temperature, which is difficult to measure directly owing to wide variation, even in a single combustion run.

$$MCE = dCO_2/ (dCO_2 + dCO) \quad \text{(Eq. 1)}$$

where dCO_2 and dCO represent the increases in concentrations of CO_2 and CO, respectively, in fire smoke compared with those in the ambient atmosphere. The range of GCR was 0.105 to 1.243, with an average value of 0.569. GCR and the gas emission factors had no clear relationship in any of the tree species tested. Differences in the physical parameters of the samples, such as porosity, density, and water content, could not explain the differences in MCE, gas emission factors, or GCR. However, MCE and the emission factor of CH_4 were negatively correlated, with a slope value of –308 ($R^2 = 0.47$), indicating that emission of CH_4 from biomass burning is controlled by combustion temperature. (K. Yagi, S. Sudo, H. Akiyama, and S. Nishimura)

Reference

Yokelson, R. J., Suscott, R., Ward, D. E., Readon, J. and Griffith, D. W. T., Emission from smoldering combustion of biomass measured by open-path Fourier transform infrared spectroscopy, *J. Geophys. Res., 102,* 18865-18877 (1997).

4) Food Production Prediction Team

The mission of the Food Production Prediction Team is to assess both the impact of global environmental change on food production and the effectiveness of technologies designed to mitigate adverse environmental changes in meeting food production targets. Our major research domains are assessment of the impact of global warming on agriculture; monitoring and modeling of environment changes in agricultural ecosystems; development of regional climate change scenarios; and assessment of the variability of climate systems in Asian monsoon countries. This year, a total of 12 researchers on the team went abroad for field surveys or presentations at international conferences, and they hosted 2 guest scientists from overseas.

In total the team has 7 activities. A new activity was initiated in FY 2004: Development of a technique to comprehensively evaluate the influence of global warming on agriculture, forestry, and fisheries. One activity was completed in FY 2004: Development of a technique for risk assessment in agro-ecosystems in light of the variability and regionality of factors that can influence these systems.

The remaining 5 activities are: 1) Development of advanced techniques for projecting future climate change by using ocean–atmosphere coupled global climate models (GCM) and statistical methods; 2) Prediction of the impacts of climate change on food supplies; 3) Prediction of agricultural productivity change, incorporating responses to global warming; 4) Evaluation of the influence of environmental change on the organic carbon content and nitrogen supply capability of soils; and 5) Evaluation of the vulnerability of agriculture by using soil, vegetation, and hydrology analyses.

***Topic:* Modified Rothamsted carbon model for paddy soils**

Soil organic matter (SOM) turnover models are very effective at simulating changes in the SOM content caused by different agricultural management systems or by climate change. However, most existing models cannot be successfully applied to paddy soils because they were developed for upland soils. SOM dynamics in paddy soils differ considerably from those in upland soils, because paddy soils are waterlogged (and therefore anaerobic) during the rice-growing period. SOM decomposition is thereby slowed, resulting in higher soil organic carbon (SOC) levels in paddy fields than in upland soils.

Paddy fields play very important roles in both food production and environmental issues in Asia. Consequently, changes in the SOC levels in paddy soils contribute markedly to changes in the calculation of CO_2 emissions from soils, as well as to changes in calculations of total national soil productivity. Therefore, an SOM model that can accurately simulate the SOC turnover in paddy soils needs to be constructed.

Of the existing SOM models, the Denitrification–Decomposition (DNDC) model has been applied to paddy soils. The good performance of the decomposition submodel of the DNDC model has been verified for long-term SOC decomposition in paddy soils as well as for upland soils. However, careful tuning of crop growth parameters is required for better simulation. The detailed information on farming management required for input

parameters is often difficult to obtain, especially in long-term experiments.

On the other hand, the structure of the Rothamsted carbon (RothC) model is simpler than those of the many other SOM models published, and the few input parameters it requires can be easily obtained. Therefore, it has the advantage of having been tested with existing data sets and being applicable over a wide area. Although this model was developed for use in non-waterlogged soils, as were most other existing models, it is possible to modify it for application to paddy soils. If this rather simple model were to be modified thus, it would be very useful for estimating carbon loss from soils, as well as for planning suitable organic matter management in paddy soils, not only on a plot scale but also on regional or national scales.

The objectives of this study were 1) to apply the RothC for long-term experiments on Japanese paddy soils and 2) to modify the model to accurately simulate changes in SOC content with time in paddy soils.

We selected 5 sites for long-term (16- to 22-year) experiments with the application of the model to paddy soils. The soil types were as follows: Gley Soils in Akita and Shimane, Gray Lowland Soils in Toyama and Mie, and Yellow Soil in Oita. Gray Lowland Soils, Gley Soils, and Yellow Soils constitute 38%, 27%, and 6%, respectively, of the total area of paddy soils in Japan.

In modeling each set of experimental data, we fixed the initial SOC content to that measured at the beginning of each experiment and then simulated the changes in SOC content with time for each management system.

Two statistical indices were employed to evaluate model performance: the root mean square error (RMSE), which represents the degree of coincidence; and the mean difference (M), which is a measure of model bias (positive values indicate consistent undercalculation of measured data, and vice versa).

It was obvious that RothC consistently underestimated the SOC content of all 9 plots (5 NPK plots and 4 NPK + straw plots) at the 5 sites. We expected this result, because RothC was developed for simulating SOC dynamics in non-waterlogged soils. Underestimation may have occurred mainly because of the slow decomposition rate of organic matter during the rice-growing period, when soils are submerged and subjected to anaerobic conditions. Decomposition of organic matter is severely inhibited under these conditions, and fermentation becomes the main form of transformation, with a markedly restricted oxygen supply.

However, in paddy soils, decomposition of organic matter might be inhibited not only during the submergence period but also throughout the remainder of the year. Fungi are the major agents that decompose coarse organic debris in upland soils. Fungi display a remarkable ability to break down the skeletal components of plant debris, such as cellulose and lignin, which normally do not decompose easily. Conversely, under the submerged conditions of paddy soils, aerobic microorganisms such as fungi cannot grow well, and thriving anaerobic microorganisms, such as bacteria, generally cannot decompose these skeletal components. This difference in the composition of microorganisms between upland soils and paddy soils may account for the difference in decomposition rates of organic matter assuming that this difference in microorganism population persists when the paddy soil dries out.

Taking into account these possible differences in decomposition rate between upland soils and paddy soils, we decided to separately change the decomposition rates of RothC for the submergence period (summer) and the period without submergence (winter). We ran the model many times with changes in decomposition rates for summer and winter, and we tried to identify the optimum combinations of values of the factors required to change the default decomposition rates. Our aim was to make the modeled SOC contents consistent with the values observed in the 9 plots at the 5 experimental sites.

We then compared the observed and predicted changes in SOC content over time by using our modified model, in which the decomposition rate was changed by 0.2 in summer (the rice-growing period) and by 0.6 in winter (the fallow period or growing period of winter crops) (Fig. 1). In these long-term experiments, the predicted values were clearly much closer to the observed values in all 9 plots at 5 sites than were the predicted values obtained with the original model.

Figure 2 shows the statistics describing the performance of the original and the modified models. RMSE in simulations with changes in decomposition rate was much lower than in simulations with the original model. This supports our assumption that the model performance could be improved by changing the decomposition rates. Similarly, the absolute values of M were much lower in simulations with the modified model, indicating that this model's performance was better than that of the original model.

We conclude that, despite certain limitations, the RothC modified for paddy soils by simple tuning of decomposition rates gave a much better performance than the original model for modeling changes in SOC content over time in Japanese paddy soils under various climatic conditions, types of soil texture, and management systems. This modified model can be used to estimate carbon loss from soils and to plan suitable organic matter

management, at least in Japanese paddy soils. It could be useful for paddy soils in other regions, but further testing under other environmental conditions and other management systems might be necessary. (Y. Shirato and H. Toritani)

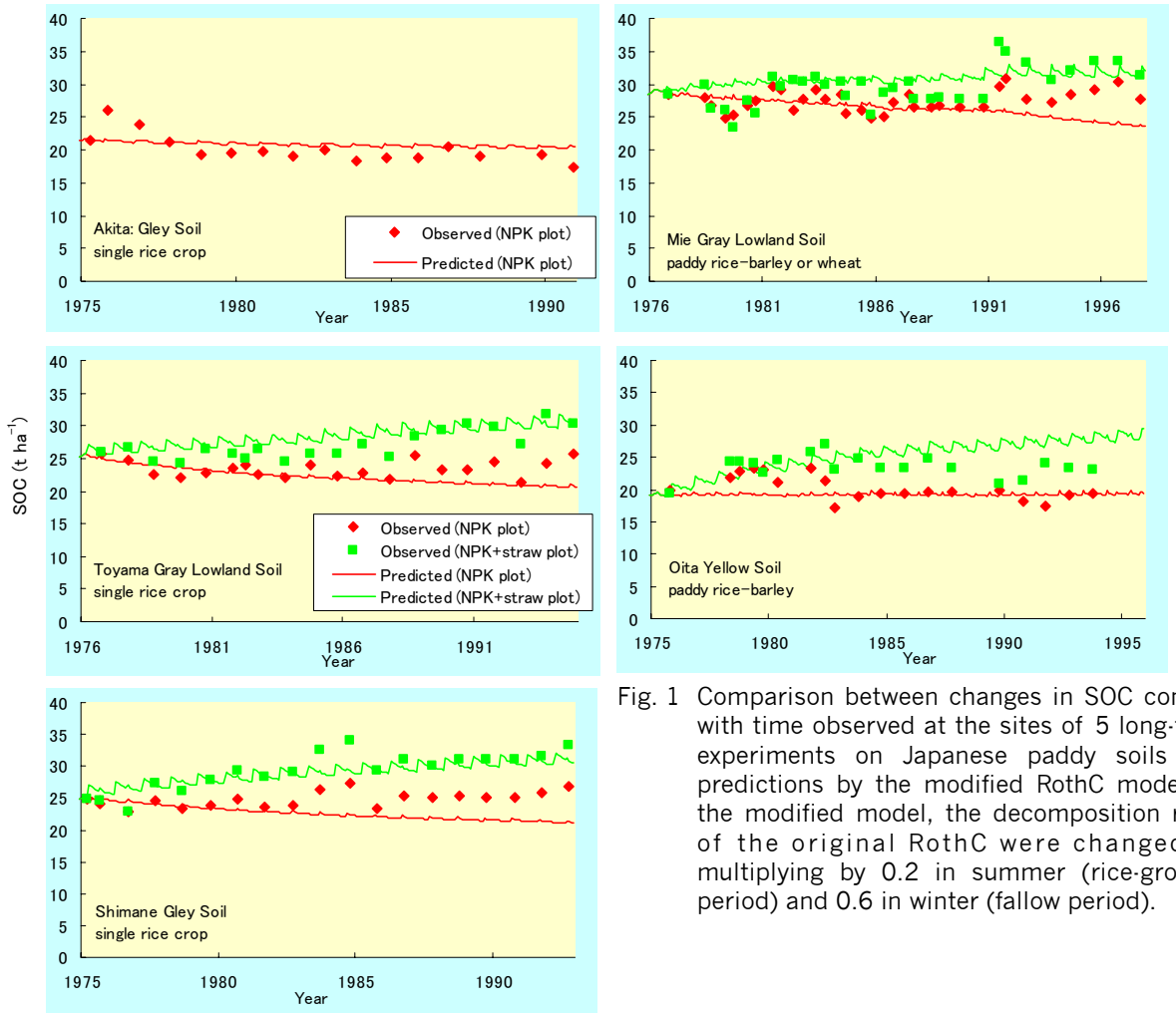

Fig. 1 Comparison between changes in SOC content with time observed at the sites of 5 long-term experiments on Japanese paddy soils and predictions by the modified RothC model. In the modified model, the decomposition rates of the original RothC were changed by multiplying by 0.2 in summer (rice-growing period) and 0.6 in winter (fallow period).

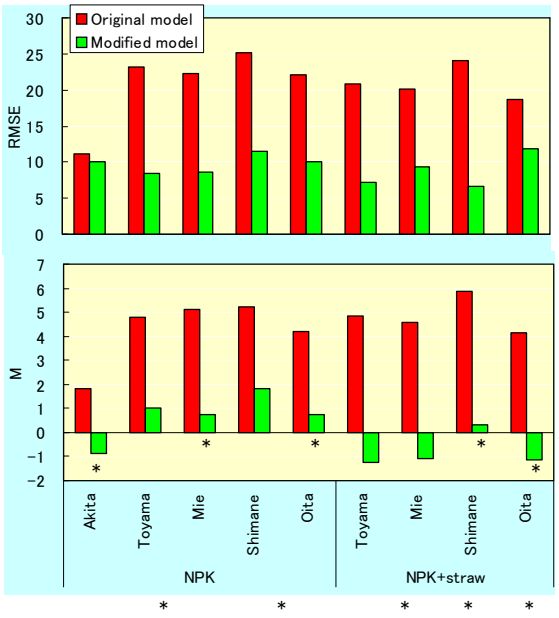

Fig. 2 Statistics describing the performance of the original RothC (red bar) and the modified model (green bar) in simulating 5 long-term experiments on Japanese paddy soils. In the modified model, the decomposition rates of the original RothC were changed by multiplying by 0.2 in summer (rice-growing period) and 0.6 in winter (fallow period). RMSE: root mean square error; M: mean difference; asterisks (*) represent absence of significant bias at $P < 0.025$ in the *t*-test for M.

5) Ecosystem Gas Exchange Team

To investigate seasonal and inter-annual variations in carbon, water vapor, and energy exchange between agricultural ecosystems and the atmosphere, the Ecosystem Gas Exchange Team conducts long-term observations of gas and energy fluxes at 3 sites: a single-cropping rice paddy field in central Japan, a natural wetland in eastern Hokkaido, Japan, and a wet sedge tundra at Barrow, Alaska. In 2004, our main focus was on the paddy site (Photo 1). Along with making standard measurements of meteorological and ecological variables, we are measuring the flux densities of carbon dioxide (CO_2), methane (CH_4), water vapor, and sensible heat by using the eddy covariance method. Process studies on carbon exchange utilizing stable isotopes are also conducted at the paddy site.

In 2004, the following measurements were added to the paddy site: 1) soil CO_2 flux throughout the year using a dynamic chamber technique; 2) stable isotope composition of CO_2 respired from, and assimilated by, the paddy; and 3) horizontal distribution of spectral reflectance of the canopy, in collaboration with the Agro-Ecological Sensing Unit, NIAES. Meteorological conditions in summer 2004 contrasted with those of the previous summer, and were characterized by the third-highest air temperature since 1946 and more solar radiation than in normal years. Under meteorological conditions favorable to rice growth, net CO_2 uptake by the paddy during the 2004 growing season was 35% greater (140 g C m^{-2} greater) than in the previous summer, and methane emission also increased by 10% (by 2 g C m^{-2}). Comparison of the seasonal CO_2 budget estimated from long-term flux data with the amount of carbon accumulated as rice dry matter indicated that about 25% of the ecosystem respiration originated from the soil, even when the paddy was flooded. The direct measurement of soil CO_2 flux using the chamber technique and the isotopic signature of CO_2 respired from the ecosystem both support the importance of the below-ground contribution to the CO_2 budget of the paddy during the growing period, although further investigations are needed for quantification.

Photo 1 The flux site at Mase, near Tsukuba in central Japan, in August. Carbon dioxide exchange between a single-cropping rice paddy field and the atmosphere is continuously monitored at this site.

Our studies are supported by MAFF and MOE (Ministry of the Environment) and are closely linked to AsiaFlux, which operates tower-based sites for the observation of carbon and water vapor exchange between terrestrial ecosystems and the atmosphere in eastern and southeastern Asia as part of the worldwide network FLUXNET. Through the activities of AsiaFlux, we are collaborating with domestic institutions such as Okayama University, the Kyushu-Okinawa Agricultural Research Center, the Forestry and Forest Product Research Institute, the National Institute of Advanced Industrial Science and Technology, and the National Institute for Environmental Studies. We are giving technical support to a new project ,"Establishment of good practices to mitigate greenhouse gas emissions from Japanese grasslands (FY 2004–2006)", organized by the Japan Grassland Agriculture and Forage Seed Association and funded by the Racing and Livestock Association. We are also collaborating with the International Arctic Research Center, San Diego State University, and Bangladesh Agricultural University. We host a post-doctoral fellow funded by MOE, and 4 technical staff members under the Cooperative System for Supporting Priority Research (FY 2000–05), sponsored by the Japan Science and Technology Agency.

***Topic:* A basic program to process eddy covariance data obtained by long-term CO_2 flux measurement**

Eddy covariance is a direct method of measuring the turbulent transport of mass and energy in the atmosphere by using a sonic anemometer and a gas analyzer. The eddy covariance method was first applied successfully to CO_2 flux measurement in about 1980, with an indispensable contribution by Japanese scientists. From the late 1990s, the eddy covariance method has been operationally employed to measure the amounts of CO_2 absorbed by, or emitted from, the world's forests, grasslands, and croplands to accumulate basic data for estimating the CO_2 budget of the global terrestrial ecosystem. In addition, the eddy covariance method has been gradually popularized in various fields as a standard method of measuring gas exchange between plant/soil and the atmosphere.

In the observation of CO_2 flux by the eddy covari-

ance method, fieldwork is now less demanding than before owing to improvements in infrared gas analyzers, but instead the data processing has become demanding. This is first because enormous data sets are accumulated by long-term observation, and second (and more important) because the data processing has become advanced and has become complicated as a result of endeavors to estimate CO_2 exchange as accurately as possible from data obtained under non-ideal conditions. Studies on eddy covariance data processing are still continuing in an effort to solve the remaining problems. The processing of eddy covariance data requires an understanding of micrometeorology. Investigators have to process data in their own ways because the data processing is still under development and depends on site-specific conditions such as topography, meteorology, and vegetation. Lack of data processing programs that can be easily used is a hurdle for investigators who are not familiar with micrometeorology and hinders further popularization of the eddy covariance method.

Under these circumstances, we produced a post-processing program that includes basic processing and the required corrections, such as coordinate rotation, frequency response correction and density correction, and which can be used by investigators who are not specialized in micrometeorology. Along with raw eddy covariance data, users prepare several instructions on data processing and site information, including the configuration of sensors, aerodynamic parameters, and supporting meteorological data. The results of executing the program include not only calculated half-hourly fluxes but also the results of various quality control (QC) tests, random errors, and flux footprints (Fig. 1). These supplementary data are useful for evaluating the reliability of the calculated fluxes, and for rejecting and removing erroneous flux data that have been influenced by instrument malfunction or inadequate meteorological conditions. Users can make practical data sets for further analysis by adding site-specific processing, if needed. Caution has to be exercised to ensure that the program does not cover gap-filling procedures. Although the filling of missing or rejected half-hourly flux data is inevitable in estimating annual CO_2 budgets, it is difficult to incorporate gap filling into general data processing programs because the appropriate gap-filling procedure is highly dependent on site-specific conditions. Incorporation of gap filling into the program is our next challenge. (A. Miyata and M. Mano)

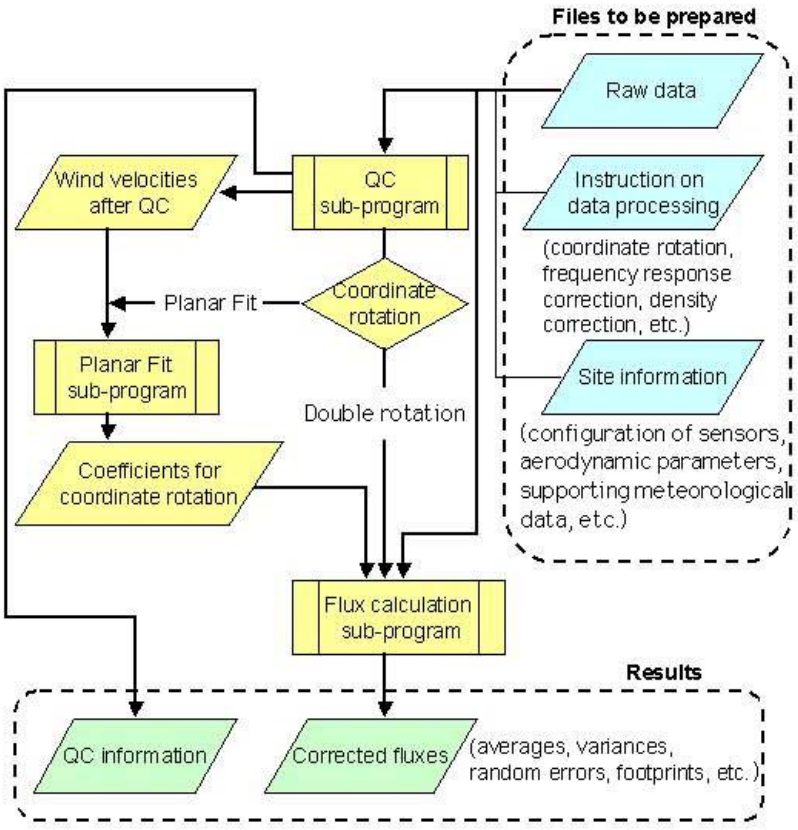

Fig. 1 Schematic diagram of a program developed for post-processing of eddy covariance data obtained by long-term flux measurements.

Department of Biological Safety

The Department of Biological Safety includes the 3 research groups of Plant Ecology, Entomology, and Microbiology, as well as the Genetically Modified Organism (GMO) Assessment Team, as described below. The GMO Assessment Team was formed with the aim of using recent advances in biotechnology and bioindustry for environmental impact assessment of GMOs. The mission of the Department as a whole is to assess the environmental impact of GMOs as well as of alien invasive and introduced living organisms, and to investigate the interaction of biodiversity and agriculture in terms of biosafety and sustainable agriculture. The Department is developing advanced methods to use in these assessments.

The major research domains of the Department are: 1) evaluation of the influence of agricultural activities on agro-ecosystems and biodiversity; 2) environmental impact assessment of introduced natural enemies and alien invasive organisms; 3) identification of biologically active chemicals and their effects on organisms in agro-ecosystems; and 4) risk assessment of GMOs within agro-ecosystems. Research is performed in collaboration with other research groups within and outside NIAES, and the approaches cover a number of research fields, such as molecular, chemical, population, and landscape ecology.

The research of the **Plant Ecology Group** is focused on vegetation dynamics and conservation of vegetation, assessment of invasive and introduced plant species in agro-ecosystems, and plant diversity in relation to agricultural production. Current research topics are: 1) effects of sulfonylurea herbicides on plant species, including aquatic plants, in agro-ecosystems; 2) landscape ecological approaches to the prediction of vegetation dynamics in relation to farmland use; and 3) the search for allelochemicals and elucidation of allelopathic mechanisms for maintaining agro-ecological vegetation.

The **Entomology Group** focuses on the following 3 major targets: 1) ecological risk assessment of alien insects such as natural enemies of insect pests; 2) analysis of the population dynamics of insect herbivores responding to the spatial distribution patterns of plants; and 3) identification of semiochemicals and analysis of the mechanisms of sex pheromone resistance.

The **Microbiology Group** aims to characterize microbial communities and to develop technologies for effective management of microbial resources in agro-ecosystems. Current research activities are: 1) investigation of microbial diversity and interactions in the soil under different agro-ecosystems; 2) analysis of the effects of environmental factors, including microbial secondary metabolites, on the survival and diversity of microbes; and 3) determination of the taxonomy, biology, and ecology of nematode communities.

Photo Participants from 8 Asian countries at the international workshop held at the Agricultural Research Institute, Taichung, Taiwan, in November 2004 to discuss the development of a biological invasion database.

The research objective of the **GMO Assessment Team** is to investigate the effects of the release of GMOs on the environment. The principal fields of interest are: 1) clarification of the dispersal and transfer mechanisms of genes from GMOs to other organisms; 2) assessment of the impact of *Bacillus thuringiensis* (Bt) toxin in corn pollen on *Lepidoptera*; and 3) monitoring of changes in the composition of weeds, insects, and soil microorganisms caused by the cultivation of genetically modified crops.

The major activities of the department in FY 2003 were: 1) publication of 11 main research results in the *NIAES Major Research Topics Annual* (these results are described below as topics in the introduction of research groups and the team); 2) organization of an international workshop on "Development of a database on biological invasion in the Asian-Pacific region" in Taiwan in November 2004, in collaboration with Taiwanese agricultural organizations, as well as the combined national meeting of the 24th Symposium on Agro-Environmental Science and 7th Seminar on Vegetation Science on 'Agricultural Use of Biological Functions for the Conservation of Agro-ecosystems; Co-actions through the Natural and Bioactive Substances'; 3) implementation and coordination of a research project on 'Assurance of the Safe Use of Genetically Modified Organisms' and participation in several projects organized by MAFF and the Ministry of Education, Culture, Sports, Science and Technology (MEXT); and 4) attendance of departmental staff at many international symposia and workshops.

Furthermore, Dr. Y. Fujii, a research unit leader in the Chemical Ecology Unit, won the Awards for his research on allelochemicals from the Japanese Society of Soil Science and Plant Nutrition in April 2004 (see Highlights).

1) Plant Ecology Group

The Plant Ecology Group consists of the **Vegetation, Landscape**, and **Chemical Ecology Units**. These units individually carry out research on the impact of agriculture on plant diversity, methods for monitoring vegetation change in agro-ecosystems, and interactions among agricultural organisms through biologically active chemicals. The major results obtained in 2004 are described below in research topics 1 to 5.

The Plant Ecology Group organized a domestic seminar on "Agricultural use of biological functions for the conservation of agro-ecosystems: co-actions of natural and active substances", held at NIAES on 10 December 2004. More than 120 people participated in the seminar, discussing the potential use and commercialization of some of the substances produced by organisms.

Topic 1: cis-Cinnamoyl glucosides as major plant growth inhibitors in *Spiraea thunbergii*

Spiraea thunbergii Sieb., a widespread ornamental plant originally from China, is used as a hedge or garden plant in Japan. This plant shows high allelopathic potential (i.e. it can inhibit the growth of other plants by the production of plant-growth inhibitory chemicals). We therefore conducted a bioassay-directed purification to isolate these plant-growth inhibitory chemicals. Two compounds were isolated as major plant-growth inhibitors, and their chemical structures were elucidated as novel *cis*-cinnamoyl glucosides, *cis*-CG and *cis*-BCG (Fig. 1, Hiradate et al., 2004b; *see Appendix for full reference*). The plant-growth inhibitory activities of *cis*-CG and *cis*-BCG were of the same strength and 2 to 4 times stronger than that of *cis*-abscisic acid (a plant hormone, Fig. 2A), indicating that the inhibitory potential of *cis*-CG and *cis*-BCG could be among the highest yet reported for natural products. *cis*-Cinnamic acid (*cis*-CA, Fig. 1), which is a component of *cis*-CG and *cis*-BCG,

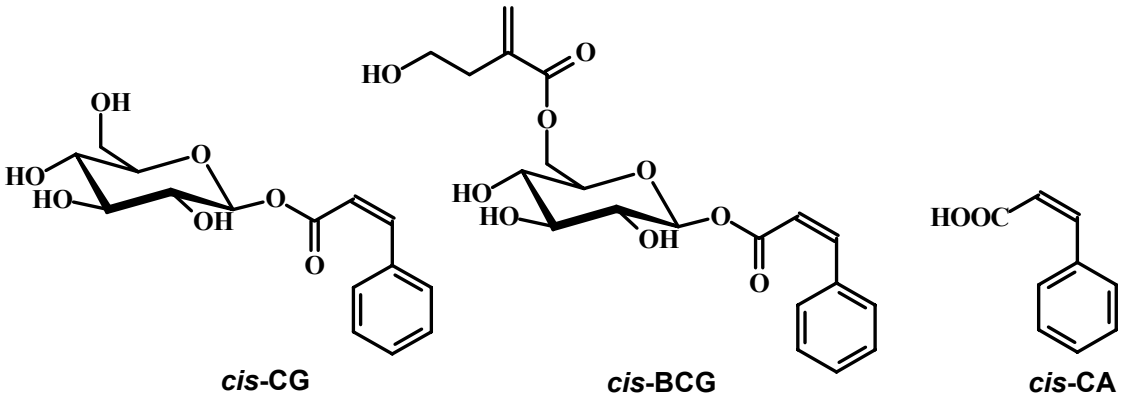

Fig. 1. Chemical structures of *cis*-CG, *cis*-BCG, and *cis*-CA.

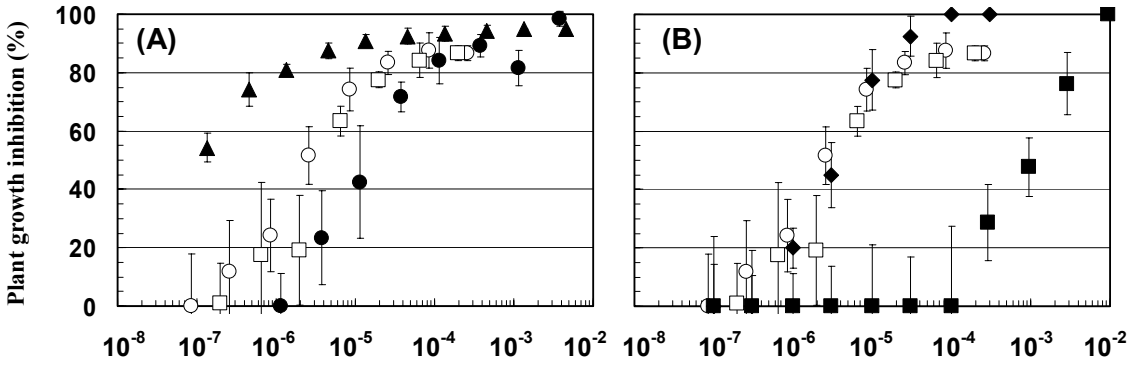

Fig. 2. Comparison of plant-growth inhibitory activities of *cis*-CG (○) and *cis*-BCG (□) with those of commercial compounds (A; 2,4-D (▲), *cis*-abscisic acid (●)) and with those of cinnamic acids (B; *cis*-cinnamic acid (◆), *trans*-cinnamic acid (■)). Test plant: lettuce (*Lactuca sativa* cv. Great Lakes 366). Bars indicate standard deviation (n = 5 or 6).

was found to possess almost the same inhibitory activity as *cis*-CG and *cis*-BCG (Fig. 2B), indicating that the chemical structure essential to the high inhibitory activity of *cis*-CG and *cis*-BCG was *cis*-CA (Hiradate et al., 2005). These findings indicate the potential roles of *cis*-CA and its glucosides as allelochemicals and their possible use as plant growth regulators in agricultural fields (Hiradate et al., 2004a). (S. Hiradate, H. Araya, Y. Fujii, H. Sugie, S. Morita, A. Furubayashi, J. Harada)

***Topic 2:* Isolation of allelochemicals from *Ophiopogon japonicus, Robinia pseudoacacia*, and essential oils from 35 domestic tree species**

We conducted laboratory and greenhouse experiments to evaluate the allelopathic potential of dwarf lily-turf (*Ophiopogon japonicus* Ker-Gawler) on lettuce, alfalfa, timothy, and rape. We investigated the effects of dry leaf debris, an aqueous extract of fresh leaves, and soil in which *O. japonicus* had been grown. The emergence, dry weight, and root and shoot length of all bioassay species were inhibited in a concentration-dependent fashion when grown in soil to which we had added oven-dried leaves of *O. japonicus*. However, the degree of inhibition varied among the test plant species. The aqueous leaf extract was highly phytotoxic and significantly reduced the germination, seedling growth, and fresh weight of all test species. The active chemicals in *O. japonicus* were isolated as β-sitosterol, *p*-hydroxybenzoic acid, and salicylic acid (*o*-hydroxybenzoic acid; Fig 3a). Of these compounds, salicylic acid was the most active and was present at a concentration of about 0.03% in the leaves; we concluded that this compound is responsible for the allelopathy.

Robinia pseudo-acacia L. known as black locust is a useful tree in temperate and subtemperate zones, but now become invasive alien plant in the central part of Japan. Vicinities dominated by this tree shows reduced growth of weeds nearby and underneath, presumably because of allelopathic interactions. Growth of both the radicles and hypocotyls of weeds (barnyard grass and white clover) and of edible plants (lettuce and Chinese cabbage) was significantly reduced when these species were grown in soil mixed with a leaf powder of *R. pseudo-acacia* at various concentrations. Aqueous leaf extracts caused significant suppression of radicle growth of lettuce and other weed species. Compounds identified from the extracts included robinetin (Fig. 3b), myricetin, and quercetin. Robinetin was the major growth inhibitor, and at 100 ppm it caused 50% growth suppression of the

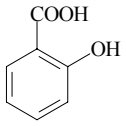

Fig. 3a Salicylic acid

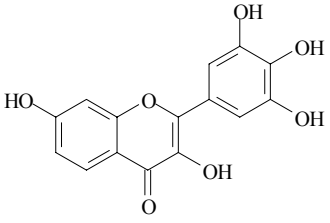

Fig. 3b Robinetin

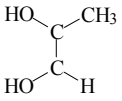

Fig. 3c 1,2-Propanediol

roots and shoots of tested plants. Myricetin and quercetin inhibited plant growth to a lesser extent. We conclude that weed decline underneath stands of *R. pseudo-acacia* and its spread into adjacent grassland vegetation results from an allelopathic interaction.

PCS (a commercial air freshener product of Field Science Co, Japan) is composed of essential oils from 35 plant species, including essential oils of Hiba (*Thujopsis dolabrata* Sieb. et Zucc.), Hinoki cypress (*Chamaecyparis obtusa* Sieb. et Zucc.), and Kumazasa (*Sasa albo-marginata* Makino. et Shibata). Chromatographic separation of a methanolic solution of PCS resulted in the isolation of a plant-growth promotive substance, which was identified by gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy as 1,2-propanediol (Fig. 3c). Seedling growth bioassay using lettuce as a test plant revealed that 1,2-propanediol acts as a plant growth promoter, and at a concentration of 0.01 mg/L it enhanced the growth of lettuce seedlings. The concentration of 1,2-propanediol in PCS was estimated as 4 g/L. These studies suggest that 1,2-propanediol is an important plant growth-promoting agent in PCS. (Y. Fujii, S. Hiradate, H. Araya, Z. Iqbal, H. Nasir, E. Nakajima)

Topic 3: **Clone distribution of hybrid dandelions on the Kanto Plain**

An examination of the plants collected in the Environmental Indicator Species Survey (Survey of Common Wildlife) by the Ministry of Environment (NIAES Annual Report, 2002) revealed that 85% of the plants identified morphologically as introduced dandelions were hybrids that had originated from crosses between native and introduced dandelions. From the viewpoint of environmental indicators, we used nuclear DNA micro-satellite markers to survey the genetic structures of hybrid dandelions collected from the Kanto Plain. Of the 263 tetraploid hybrids, 246 (93%) were classified as genetically identical clones. This dominant clone was widely distributed on the Kanto Plain. (H. Shibaike, Y. Kusumoto, T. Ohkuro, M. Ide)

2) Entomology Group

The mission of the Entomology Group is to prevent the disturbance of agro-ecosystems by native and exotic insect species, and to assess the non-target effects of introduced insects. In FY 2004, the 3 units of the division studied 3 major subjects covering 8 practical research subjects. Furthermore, the Entomology Group conducted cooperative studies with the Food Production Prediction Team, the GMO Assessment Team, and the Plant Ecology Group.

The **Introduced Insect Assessment Unit** compared the host range, sex ratio, and fertility of *Dacnusa sasakawai*, an endoparasitic wasp of the leafminer, with those of an introduced endoparasitic wasp, *Dacnusa sibirica*. We recognized no significant difference between them, so we could not obtain ecological proof as to why the introduced parasitoid prevailed over the native parasitoid. We also examined in detail the hybridization rates among introduced and native parasitoids of the chestnut gall wasps *Torymus sinensis* (introduced parasitoid) and *Torymus beneficus* (native one) by using mitochondrial and nuclear DNA markers. Hybrid posterity between *T. sinensis* and *T. beneficus* was detected within 5% of total offspring. A laboratory experiment for assessment of the non-target impact of the introduced green lacewing *Chrysoperla carnea* on the native species *Chrysoperla nipponensis* was conducted to determine rates of larval interspecific predation. (Topic 1). To accumulate and share data on invasive alien species in the Asia–Pacific region, we developed the Asian-Pacific Alien Species Database and made it available on the Internet (http://apasd-niaes.dc.affrc.go.jp/). Furthermore, a mechanistic model for describing the dispersal distance of organisms was developed by generalization of the Brownian motion model, allowing stochastic fluctuations of step length (Topic 2).

The **Population Ecology Unit** studied the effects of the spatial distribution of host plants on the population dynamics of the ragweed beetle *Ophraella communa.* We measured the food consumption of this beetle and revealed that ovipositing females consumed the greatest amounts of food. We also investigated the effect of food conditions on the dispersal of beetles from the hosts by flight. We developed a simulation model for analyzing the population dynamics of this insect, incorporating factors such as the spatial distribution of host patches and the rate of dispersal of beetles between host patches. The results of the simulation fitted the occurrence of the insects on host patches in the field.

By selection of individuals, the **Insect Semiochemical Unit** studied and established a strain of the smaller tea tortrix moth *Adoxophyes honmai* resistant to communication disruptants containing the sex pheromone (Z)-11-tetradecenyl acetate. The cause of the resistance was investigated in males of both strains (the resistant and susceptible strains) that were set in the stream of the smell of (*Z*)-11-tetradecenyl acetate; their antennae were then sprayed with synthetic pheromone containing 4 components. The antennae of males of the resistant strain showed significantly higher response (as measured by electroantennogram) than those of the susceptible strain. Furthermore, we studied the chemical structure of

the substance that is released from the stink bug *Eysarcoris lewisi* which causes pecky rice; we found that the substance was an alcohol with the chemical formula $C_{15}H_{24}O$.

The **Insect Gene Bank Project** was implemented by the above-mentioned 2 Units and the Insect Systematics Laboratory. This project started in 2000. The purposes of this project are to collect and rear successively insect species or strains, such as natural enemies or other insects, for uses such as bioassays, and to supply these insects to laboratories requesting them for research. In 2004, two aphid species, *Rhopalosiphum padi* and *Acyrthosiphon pisum*, were added to the collection. The brown planthopper *Nilaparvata lugens* (a strain virulent to a resistant rice carrying the *bph-4* gene) was added to the active collection (i.e. the collection of insects that are available for supply). The physiological and ecological characteristics of about 42 items in the collection were evaluated.

Topic 1: **Laboratory experiment to assess the non-target impact of the introduced green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on the native species *C. nipponensis* (Okamoto): larval interspecific predation**

The green lacewing *Chrysoperla carnea* (Stephens) is frequently used for biological control. It has long been assumed to be a single species that is morphologically identical throughout a Holarctic distribution range. However, more recent evidence suggests that it is not a single species, but instead a complex of several or many biological species characterized by different male courtship songs (Henry et al., 1993; 2001). In Japan, the native green lacewing is widely distributed and has been classified as *C. carnea* (Tsukaguchi, 1985). However, the name was revised to *C. nipponensis* (Okamoto) by Brooks (1994) on the basis of external morphological differences such as the color of the gradate crossveins, which are black in *C. nipponensis* and green in *C. carnea*. Its courtship song also differs from those of other species of the *carnea* group (Henry and Wells, 2004; Taki et al., 2005). In 1996, a green lacewing designated as *C. carnea* was imported from Germany on a test basis. It was registered as a biological pesticide in 2001 and is now on the market in Japan. Its gradate crossveins are primarily green.

The 2 species can now encounter each other in the same habitat. Serious concerns over the non-target impact of introduced exotic natural enemies in native ecosystems have been raised by a number of prominent ecologists and conservation biologists (Follett and Duan, 2000; Wajnberg et al., 2001; Louda et al., 2003). As part of a risk assessment of the non-target effects of the introduced green lacewing in native ecosystems, I performed a laboratory experiment to characterize the symmetry of interspecific predation between the introduced green lacewing *C. carnea* and the native closely related species *C. nipponensis* at different life stages. Older and larger larvae always ate younger and smaller individuals, regardless of species. When larvae of the same instar and similar size were paired, almost equal predation rates between the two species were observed (Fig. 1). These results suggest that size was the most important determinant of the symmetry of interspecific predation between *C. carnea* and *C. nipponensis*. Populations of *C. nipponensis* will not be decreased by inter-

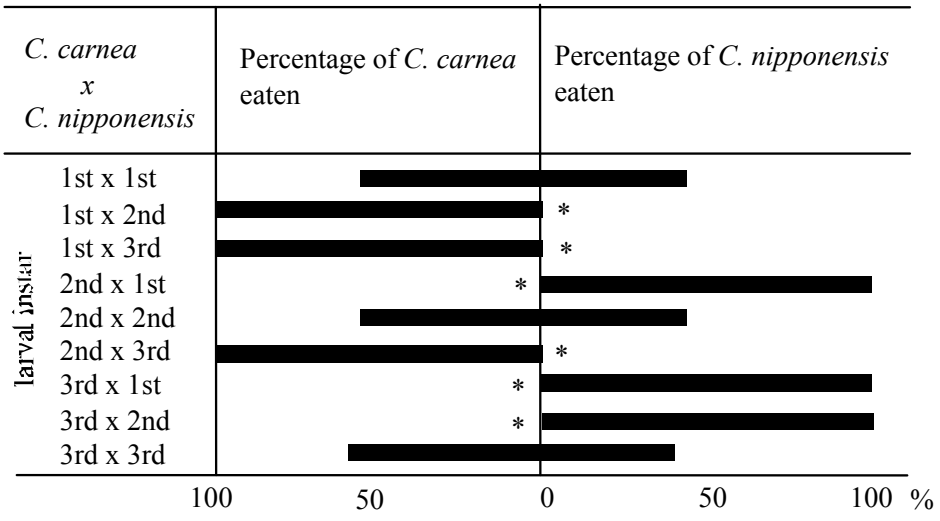

Fig. 1 Interspecific predation between introduced *Chrysoperla carnea* and native *C. nipponensis* larvae. * represents significant difference from 50% predation in each pair by X^2 test ($P < 0.01$).

specific predation with *C. carnea* unless there are extreme mass releases of *C. carnea* over a small arena (A. Mochizuki).

***Topic 2:* A new mechanistic model for describing dispersal distances of organisms by a generalization of the Brownian motion model**

The United Nations declared 2005 to be the International Year of Physics, because this year is the centenary of the seminal scientific discoveries by Albert Einstein that form the basis of modern physics. Einstein published 3 breakthrough papers in 1905: (1) special theory of relativity, (2) photoelectric theory, and (3) Brownian motion theory. The former two theories have been subsequently developed mostly within the field of physics, whereas the Brownian motion model has been applied to wider fields, including biology and economics. The Black-Scholes model, which predicts option prices, was developed by applying the Brownian motion model to the fluctuation of stock prices, and Myron Scholes won the Nobel Prize in 1997 for this work. However, it is now widely recognized that the Black-Scholes model cannot describe the fluctuation of stock prices in the real world. Stock prices fluctuate more sharply than are predicted by the Brownian motion model. A similar problem is also recognized in the field of biology. Real plants increase their ranges of distribution at speeds much faster than expected from the Brownian motion model. This problem is traditionally called "Reid's paradox".

The model developed by Einstein cannot describe actual fluctuations, so what is the difference between the Brownian motion model and the real world? The Brownian motion model seems quite comprehensive, and it includes the spatial heterogeneity of dispersing particles. However, probably for mathematical reasons, Einstein did not consider the temporal heterogeneity of dispersing particles. Yamamura (2004; Population Ecology 46: 87–101) improved the Brownian motion model by including temporal heterogeneity and derived a solution in an explicit form. This model is called the Gamma model, because a generalized gamma distribution was used in describing temporal heterogeneity.

The Gamma model can describe the real world quite well. An example of its application is shown in Figure 2,

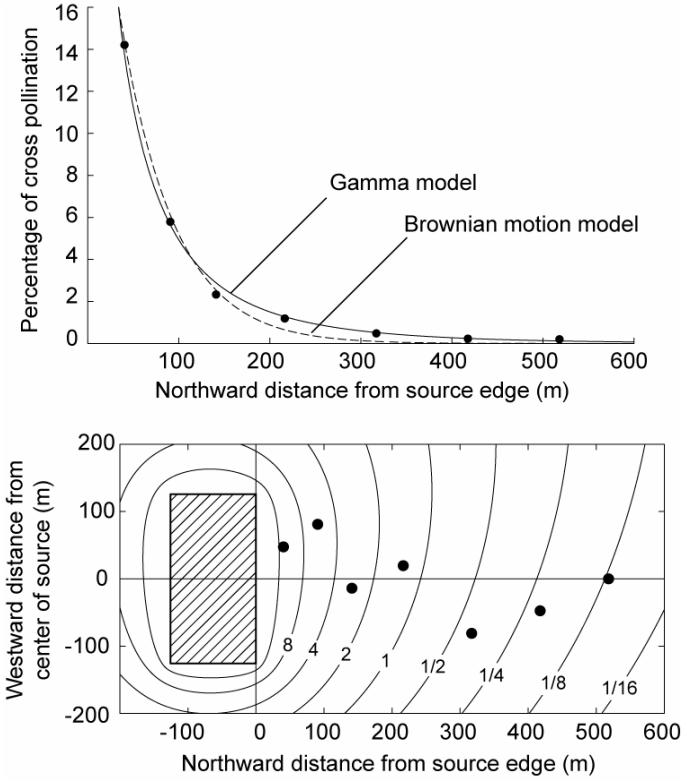

Fig. 2 Diffusion of corn pollen. Data are from Jones and Brooks (1950; Okla. Agr. Exp. Stn. Tech. Bull. T-38:1–18). *Upper panel:* Black dots indicate observed percentages of cross-pollination. Solid curve is from Gamma model. Dotted curve is from Brownian motion model. *Lower panel:* Estimated contours of percentages of cross-pollination. Black dots indicate spatial positions of samples used to estimate parameters. Hatched rectangle indicates the pollen source. (Copyright: Society of Population Ecology and Springer-Verlag).

where the spatial diffusion of corn pollen is described. The solid curve indicates the curve of the Gamma model, whereas the dotted curve indicates the curve of the Brownian motion model. The model parameters were estimated by a quasi maximum likelihood method in both cases. The Gamma model can describe long-range dispersal where the Brownian motion model fails. Thus, the Gamma model can explain Reid's paradox of plant dispersal. (K. Yamamura)

3) Microbiology Group

The Microbiology Group aims to characterize microbial communities in agro-ecosystems and develop technologies for effective management of microbiological resources. The group consists of a Microbiology Research Coordinator and 3 research units: Microbial Ecology, Microbial Genetics and Physiology, and Nematology and Soil Zoology.

Work is conducted on the following research themes: 1) investigation of the effects of soil microorganisms on the population dynamics of sclerotium-forming fungi; 2) investigation of the effect of secondary metabolites from microorganisms and plants on the multiplication of microorganisms; and 3) genus- or species-level analysis of soil nematode communities in and around upland fields, and investigation of the biological characteristics of entomopathogenic nematodes. The following activities were completed in FY 2004

The Microbiology Research Coordinator coordinated research and registration of microorganism genetic resources for related laboratories, which act as sub-banks for the MAFF Genebank system.

The **Microbial Ecology Unit** is investigating the effects of soil microorganisms on the population dynamics of sclerotium-forming fungi. *Trichoderma* spp. are associated with, and often antagonize, *Rosellinia necatrix* and *Helicobasidium mompa*, which are root pathogens of fruit trees. The concomitant presence of *Gliocladium catenulatum* is known to suppress the antagonism of *Trichoderma* spp. to *R. necatrix*, but microscopic observations failed to reveal the process of suppression. *G. catenulatum* did not antagonize *R. necatrix* on potato dextrose agar plates. Populations of fungi and bacteria in soil infested with *H. mompa* recovered soon after fumigation with chloropicrin, but addition of fluazinum diminished the fungal population. Use of the soil cover culture method revealed that treatment with these fumigants nullified fungistasis in the soil. PCR-DGGE of soil DNA enabled us to monitor changes in the microbial community in a field treated by soil fumigation. These methods are useful to evaluate the effects of artificial disturbance on soil microflora (see Topic 1). Use of a fluorescence *in situ* hybridization technique with specific probes that we developed revealed the presence of a bacterium, *Pantoea* sp., on the sclerotia of *Sclerotium rolfsii*, in masses on the hyphae.

The **Microbial Genetics and Physiology Unit** found that alginate gelation of wheat seeds, together with treatment with antagonistic bacterial strains (fluorescent pseudomonads) against *Gaeumannomyces graminis* var. *tritici*, suppressed take-all of wheat more effectively than direct treatment of seeds with antagonistic strains alone. The method was suggested to be useful in increasing the fixation of antagonistic strains to the plant's roots. To understand the colonization and its relationship to disease suppression, we engineered the antagonistic strain to express a green fluorescent protein (Gfp) constitutively, and we then used the green fluorescent strain to analyze the behavior of the antagonistic strain on wheat seeds and roots. The antagonistic strain moved along the germinating root(s) from the seed and colonized them. Such green fluorescent strains should be powerful tools for analyzing the behavior of microorganisms introduced into the rhizosphere.

We investigated the genotypic identification and characterization of *Burkholderia cepacia* complex (Bcc) strains recovered from clinical and environmental sources. On the basis of 16S rDNA RFLP analysis, Bcc strains derived from clinical sources were assigned to *B. cepacia* genomovar 1, *B. cenocepacia, B. stabilis,* and *B. vietnamiensis*. In contrast, the majority of Bcc strains from environmental sources belonged to *B. cepacia* genomovar 1, whereas the rest belonged to *B. cenocepacia*.

We are developing a PCR method for use in quantitative analysis of a recombinant soybean root nodule bacterium (*Bradyrhizobium japonicum*) in soil. First, we designed primers/TaqMan probe on the basis of the sequence of the marker gene integrated onto the chromosome of the target strain; this sequence proved to be highly strain-specific. Furthermore, by using a bead-beating method with the addition of skim milk, we succeeded in extracting from the soil DNA that was suitable as a template for quantitative PCR.

To clarify the relationships among the *Agrobacterium/Rhizobium* complex and their relatives, we tried to select the indices required for molecular phylogenetic analysis. We selected 4 single-copy, indispensable genes, *recA*, *atpD*, *dnaK*, and *rpoD*, as index candidates. Using the respective genes, we preliminarily constructed phylogenetic trees, all of which showed similar results in regard to topology at a family level.

The **Nematology and Soil Zoology Unit** identified free-living soil nematodes in a no-till manure-amended field. Identification of bacteriophagous nematodes be-

longing to the order Rhabditida has been almost completed, with the detection of the genera *Deontolaimus* and *Oigolaimella*. Identification of fungivorous nematodes belonging to the order Tylenchida bore the genera *Filenchus* (Tylenchina, Tylenchidae) and *Ditylenchus* and *Safianema* (Anguinidae). A nematode belonging to the genus *Aphelenchoides*, which has a unique tail-end shape, had presumably never previously been described.

From our investigation of the biological characteristics of fungivorous nematodes, we ascertained that *Tylencholaimus parvus*, belonging to the order Dorylaimida, is a real fungivore that replicates in some fungal cultures. Its growth rate in the culture of 9 species of fungi was significantly lower than that of *Aphelenchus avenae*.

To find indices suitable for evaluating the diversity or function of soil nematodes, we analyzed nematode biodiversity data from a no-till manure-amended field and the results of an experiment to determine the effects of fumigants on nematodes.

***Topic 1:* Impact of soil fumigation on microbial communities, as revealed by a culture-independent molecular method**

Soil microorganisms have important roles in agriculture. They are responsible for the mineralization of organic matter, whereby nutrients are recycled and pollutants are degraded, as well as for the suppression of soil-borne diseases. The impact of soil fumigation on microbial communities has been an issue of concern to soil microbiologists. Traditionally, investigations of soil microbial community have been based mainly on culture experiments. These methods are both time- and labor-consuming and are applicable to the fewer than 1% of microorganisms present in the soil that can readily be cultured. Recent improvements in methods of extraction of DNA from the soil have facilitated molecular analyses of microbial communities, including of unculturable microorganisms.

We used a culture-independent, molecular method to study the impact of fumigants such as chloropicrin and 1,3-dichloropropene (D-D) on the soil microbial community in an experimental plot at NIAES over 2 years. DNA was directly extracted from the soil, and the bacterial community structure was studied by 16S rDNA PCR-DGGE. Prominent DGGE bands were excised and sequenced to gain insight into the identities of the predominant microbes. Bacterial community analysis revealed that the majority of band sequences from unfumigated soil samples were most closely related to the sequences of unculturable bacteria (Fig. 1). After chloropicrin treatment, these bands became undetectable, and other bands, which showed high sequence homology to those of culturable bacteria, increased in intensity (Fig. 1). These results indicated that unculturable bacteria are more drastically affected by soil fumigation.

The DGGE patterns from unfumigated, control soil showed no temporal fluctuation. In contrast, 1 month after the first fumigation the DNA level in chloropicrin-treated soil decreased and the DGGE pattern changed dramatically. Even 1 year after fumigation, neither the

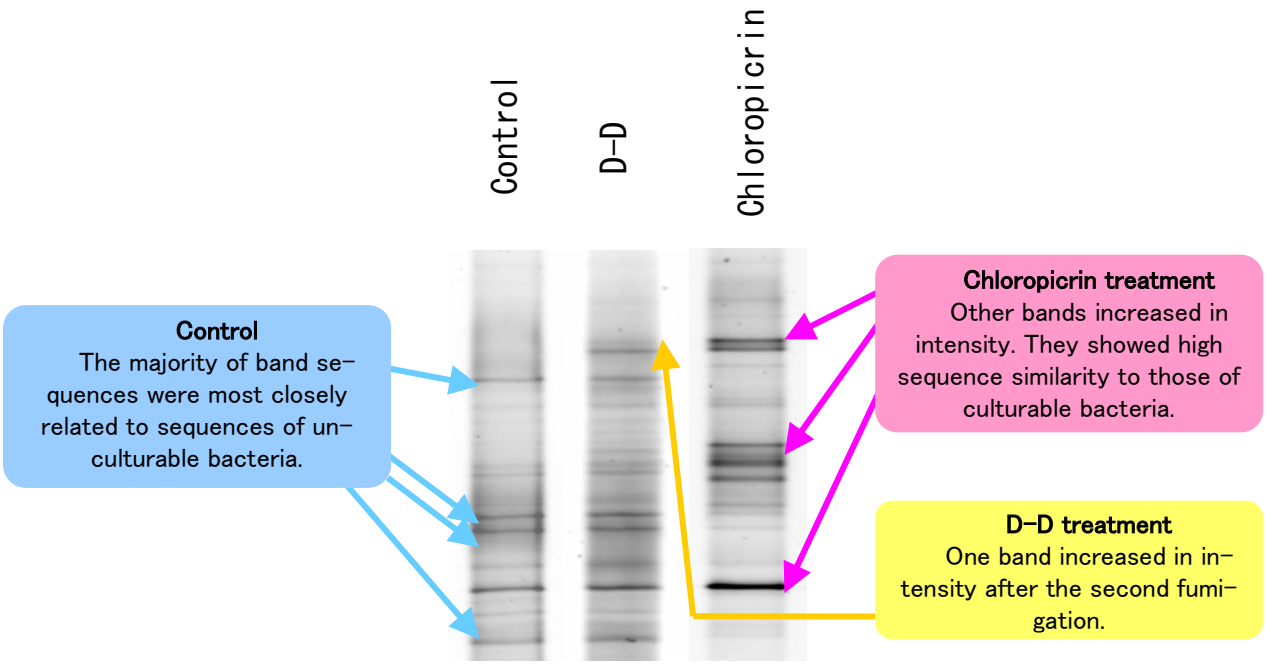

Fig. 1 16S rDNA PCR-DGGE patterns after fumigation with D-D and chloropicrin

DNA level nor the DGGE pattern had recovered. In soil treated with D-D to eradicate nematodes, the DNA level and DGGE pattern were the same as those in unfumigated, control soil, except that 1 band increased in intensity after the second fumigation (Fig. 1). The results imply that D-D has less impact on the microbial community than does chloropicrin.

Direct soil DNA extraction and subsequent PCR-DGGE are useful for assessing the impact of fumigation on bacterial communities. We can also analyze fungal communities by using the same DNA samples as used for bacterial communities, because soil DNA theoretically contains DNA from all organisms in the soil. We expect that this culture-independent method will be applicable to other impacts, such as the effects of chemical pollution and of organic matter application on soil microbial communities. (Y. T. Hoshino)

Topic 2: Influences of soil fumigation with agrochemicals on free-living soil nematode populations and their recovery.

Of all the activities performed on farms, fumigation with agrochemicals such as chloropicrin has the greatest influence on soil-inhabiting organisms. It is well known that these fumigants reduce the numbers of target soil-borne microorganisms or plant-parasitic nematodes, but there have been few reports on the effects that non-target soil-inhabiting animals suffer from fumigation. No reports have shown the long-term effects of fumigant application on soil animals or the periods needed for free-living nematode populations to recover their density before the next application. We designed an experiment to determine the effects of chloropicrin, D-D, and methyl bromide on free-living nematodes in microplots at NIAES in 2001 and 2002. Soil fumigated with chrolopicrin or D-D, together with untreated control soils, were arranged in nine 5 × 6 m microplots in a 3 × 3 Latin square design. Soil fumigation was done late in September in both years. In 2002, fumigation of the surrounding space with methyl bromide was added. Two weeks after treatment, each plot was cultivated to volatilize the fumigants and then seeded with spinach. Spinach cultivation was repeated to give 2 crops a year, with the products turned over. Four soil samples were taken from each plot before fumigation, just after fumigation, and 1, 6, 9, and 12 months after fumigation. Nematodes in 20 g soil were extracted by the Baerman-funnel method and counted to each distinguishable taxon under a compound microscope with a magnification of ×100. Figure 2 shows the changes in numbers of species after each fumigation treatment. The number of species in the control plots was about 20 and stable, whereas numbers in the fumigated plots were drastically diminished to below 5. Species numbers in the fumigated plots showed a gradual recovery to about 15, less than in the control plots. In the fumigated plots the total numbers

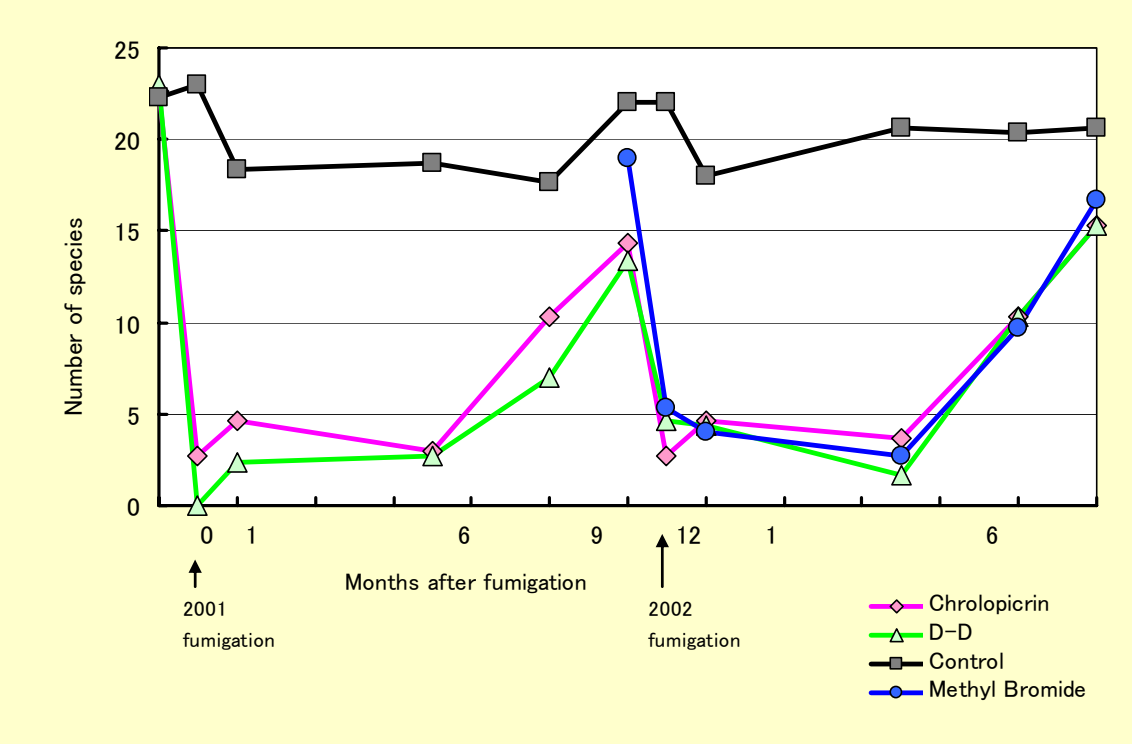

Fig. 2 Changes in numbers of species after each fumigation treatment.

of nematodes detected were also reduced, to 13/100 g wet soil, compared with more than 1000 in the control plots. However, total number of nematodes in the fumigated plots sometimes surpassed that in the control plots 9 months after treatment and was quicker to recover than number of species (data not shown). Nematodes belonging to the genus *Aphelenchoides* (fungivores), the order Rhabditidae and the genus *Acrobeloides* (bacteriovores) were the main components of the nematode population after recovery. On the other hand, nematodes belonging to the genera *Filenchus* (fungivore), *Heterocephalobus*, *Cerevidellus* (bacteriovores), and *Opisthodorylaimus* (omnivore) decreased in number after recovery. The drastic influence of fumigant application on free-living nematodes in the soil was clearly shown here, indicating that nematode community structure changes after fumigant application. (M. Araki)

4) GMO Assessment Team

The missions of the GMO Assessment Team are: 1) to clarify the ecological impact of GMOs (genetically modified organisms); 2) to develop standards of risk assessment for GMOs; and 3) to collect basic information and documents relating to GM and conventional crops. The major results of our research projects are described as follows.

A field experiment was conducted to monitor the changes in composition of weeds, insects, and soil microorganisms in response to long-term GM crop cultivation. The aim of this research project is to evaluate the environmental impact of continuous cropping of GM crops for 3 to 5 years. We conducted monitoring experiments on GM crops of maize, rice, canola, and soybean at 4 national agricultural research institutes and centers to clarify the effects of these GM crops on organisms. As part of these experiments, we have cultivated glyphosate-tolerant (GMO) and conventional (non-GMO) soybean cultivars in summer and glyphosate-tolerant and conventional canola cultivars in winter in a 0.2-ha experimental field since 2001. Weeds are controlled by glyphosate application in the GM experimental plots and by intertillage in the control plot. We have investigated changes in vegetation and in the composition of insect and soil microorganism populations over the past 4 years. The cultivation of GM soybean and canola will be followed by that of conventional wheat and soybean on the same experimental fields next winter and summer, respectively, to evaluate the environmental effects of long-term GM crop cultivation.

Pollen flow and outcrossing rate were investigated in 2 commercial sweet corn cultivars with yellow and white grains, which were pollen donors and pollen recipients. The outcrossing rate was determined by the xenia phenomenon, which appeared on the ears of the white recipient corns. We have examined the outcrossing rate within 50 m of the pollen donor plants for 5 years since 2001, and in 2002 we also initiated collaborative research work between NIAES and the Tsumagoi Station of the National Center for Seeds and Seedlings (NCSS) to conduct large-scale experiments in a 4.5-ha (100 m × 450 m) field. Outcrossing rates and distributions of hybrid plants have been different each year. However, recipient plants within a distance of 1 m from the yellow donor plants showed a mean outcrossing rate of 49.6% over 4 years at Tsukuba, and 43.6% over 3 years at Tsumagoi. There were no large differences between the 2 experimental sites in terms of the mean outcrossing rate of recipient plants neighboring the donor plants. These rates decreased sharply with increasing distance from the donor plants. In the small-scale field at Tsukuba, the mean outcrossing rate over 4 years decreased to 0.2% at a distance of 50 m. On the other hand, the mean rates over 3 years decreased to 2.3% at 50 m, 1.3% at 100 m, 0.7% at 200 m, and 0.1% at 400 m in the large-scale field at Tsumagoi. At the Tsumagoi Station we also performed a field experiment to see if we could decrease the outcrossing rate of corn by using a windbreak net.

We have been monitoring the distribution and weediness of GM canola (*Brassica napus*) around the Kashima Seaport, which is one of the ports in Japan where canola seeds are unloaded (Fig. 1). We have also conducted further investigations into the transfer of introduced herbicide-tolerant genes to relatives of *B. napus* growing in the area. (K. Matsuo)

Fig. 1 Feral canola (*Brassica napus*) growing by the side of a road.

Department of Environmental Chemistry

Year-round production of large quantities of high-quality agricultural products is associated with repeated heavy loading of farmland with fertilizers, pesticides, and livestock wastes. This, in turn, leads to air, water, and soil pollution with substances such as pesticides, nitrates, and heavy metals. With increasing combustion of refuse, lethal dioxins are being released to the environment. The Department of Environmental Chemistry has a mandate for food security and ecosystem conservation against a number of farm chemicals from the 3 broad standpoints of "risk assessment", "risk reduction", and "environment remediation".

The Department consists of 3 research groups and 1 team corresponding to the chemicals targeted, namely: 1) a group researching organic chemical compounds such as farm chemicals; 2) a group researching heavy metals (in particular, cadmium); 3) a group researching nutritional salts, such as nitrogen and phosphate; and 4) the dioxin research team. Each group has a leader and several research units. The major research fields of each group are described below.

Organochemicals Group: Pesticides play a vital role in food security and will remain indispensable unless more effective and less risky replacements can be developed. There is much concern about the ecotoxicity of pesticides in air, water, and soil from farmlands. This group is responsible mainly for the development of innovative and sophisticated technologies for studying the influence of pesticides on the environment and how to decrease the amounts of chemicals used. Major research topics are: 1) the dynamics of pesticides in soils, water, and the atmosphere; 2) risk assessment of pesticides in aquatic organisms such as algae, aquatic midges, and medaka fish (killifish); 3) development of environmentally friendly crop protection systems, and 4) development of technologies for the bioremediation of recalcitrant organic compounds, involving a) molecular genetics and genetic diversification of bacteria that degrade chlorobenzoates, PCBs, and 2,4-D, b) *in situ* bioremediation of soils contaminated with recalcitrant organic compounds, and c) risk assessment of recombinant bacteria.

Heavy Metal Group: The Codex Committee, established jointly by FAO and WHO, has been developing a new international safety standard for cadmium in foods to minimize its human intake. Under such circumstances, it is a matter of urgency that we elucidate the behavior of heavy metals in soils and the mechanism of their absorption by crops, and that we develop technologies to suppress hazardous metal absorption by crops. The Heavy Metal Group has 3 ongoing research projects: 1) evaluation of heavy metal loadings in arable soils and elucidation of the mechanisms of their absorption by crops; 2) elucidation of the chemical forms of heavy metals in soils and development of technologies for suppression of their absorption by crops; and 3) determination of differences in the abilities of various staple crops to absorb heavy metals.

Water Quality and Solute Dynamics Group: Recently, public concern has risen over the contamination of various river basins and lakes by nutrient solutes such as nitrate nitrogen and phosphate. Since the implementation of new regulations against NO_3-N and NO_2-N contamination began in 1999, a number of agricultural activities have been placed under strict surveillance to ensure that NO_3-N and NO_2-N levels in groundwater do not exceed the critical concentration of 10 ppm. There is an urgent need to formulate an effective solution for this problem. There are 3 ongoing projects in this group: 1) study of the dynamics of nitrate nitrogen and other nutrient solutes in soils and small- and medium-sized watersheds; 2) development of methods for monitoring levels of nutrient solutes in medium-sized river basins; and 3) evaluation of methods for enhancing the denitrification capabilities of natural mass flows and development of technologies for alleviating negative loadings of nutrient solutes.

Dioxin Dynamics Team: Contamination of agricultural products with dioxins has become a serious concern for both consumers and producers. There is an urgent need for the production of dioxin-free agricultural products. In this regard, there are 2 ongoing projects: 1) study of the dynamics of dioxins in crops and farmland, and 2) development of technologies for the physico-chemical and biological decomposition of dioxins.

1) Organochemicals Group

The missions of this group are to assess and reduce the environmental risk caused by application of pesticides and persistent organic pollutants (POPs) in agro-ecosystems and to develop bioremediation techniques to restore environments contaminated with recalcitrant organic chemicals.

In FY 2004, the group studied the following major research subjects: 1) risk assessment of pesticides in aquatic organisms; 2) multimedia modeling to predict the fate of POPs; 3) mechanism of induction of systemic acquired resistance in plants by some organic chemicals; 4) molecular genetics and molecular ecology of bacteria that degrade chlorobenzoates and 2,4-D in soils; 5) *in*

situ bioremediation of pesticide-contaminated soil by using charcoal enriched with degrading bacterial consortia.

Sixteen original research papers were published this FY. In September 2004, the group organized the fourth Seminar on Organic Chemicals Studies: Mechanisms of POP Persistence in Soil and the Technology of Risk Reduction and Principles and Utilization of Soil Adsorption, and the 21st Research Meeting on Pesticides: Environmental Risk Management of Drift in Pesticide Application.

The **Environmental Pesticide Assessment Unit** developed a system for the indoor breeding of the caddisfly (*Cheumatopsyche brevilineata*) to assess the effects of pesticides on aquatic invertebrates in inland water ecosystems (Topic 1). The caddisfly was selected as an insect representative of those in Japanese rivers—that is, as a key species. By bioassays using native algal species such as diatoms, the Unit clarified the effects of herbicides on algal production and observed a wide range of susceptibility to certain herbicides in the middle reach of the river, where many paddy fields are located. For POPs, the Unit is developing a prototype multimedia model to clarify how POPs are emitted and diffused from Asian regions.

The **Pesticide Mitigation Unit** met the challenge to develop an alternative chemical—a resistance inducer or "plant vaccine"—for the control of fungal plant diseases, which currently relies largely on the use of ordinary fungicides. As an alternative chemical, the Unit selected acibenzolar-*S*-methyl (ASM) and studied the mechanism of long-lasting induction of systemic resistance in plants. In ASM-pretreated cucumber plants, the gene encoding callose (β-1,3-glucan) synthase was highly expressed after fungal attack, resulting in physical blockage of pathogen penetration and development. The longevity of systemic resistance induced by ASM was well demonstrated in cucumber under greenhouse condition. Fewer spray applications of ASM than of ordinary fungicides still maintained a high level of protection against powdery and downy mildews on cucumber. In addition, the Unit used the sequence difference in a β-tubulin gene to identify the *Fusarium* species that cause head blight on cereals. The PCR-Luminex system was successfully applied for this purpose. Furthermore, biological monitoring studies showed that isolates resistant to strobilurin fungicides are widely distributed in Japan, irrespective of the usage history of this class of fungicides. Molecular characterization of the resistance mechanism is under investigation at present. By designing a pair of forward and reverse primers related to carboxylesterase, the Unit also developed a simple gene diagnosis method for specific qualitative detection of strains of cotton aphids resistant to organophosphorus (OP) insecticides (Topic 2).

The **Applied Soil Microbiology Unit** studied the mechanisms regulating expression of the chlorobenzoate and 2,4-D degradative genes of various soil isolates and the chitinase genes of *Streptomyces* spp.. A study of chimeric mutants of chlorocatechol 1,2-dioxygenases revealed several amino acid residues responsible for the differences in substrate specificities. The Unit also analyzed the characteristics of plasmids with 2,4-D degrading genes. 3-chlorobenzoate (3CB)-degrading bacteria whose concentrations increased in a forest soil after addition of 3CB were detected by a culture-independent method, polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE). Furthermore, to develop *in situ* bioremediation of pesticide-contaminated soil, bacterial consortia decomposing both quintozene (PCNB) and simazine were constructed in charcoal. The simazine-decomposing bacterial consortia were found to be composed of *Arthrobacter* spp., *Bradyrhizobium japonicum,* and a novel strain of β-*Proteobacteria.*

Topic 1: System for indoor breeding of caddisfly (*Cheumatopsyche brevilineata*) for ecological toxicity assessment

Japan's current pesticide registration system requires us to perform a set of acute toxicity examinations in 3 species—fish (e.g. carp or medaka, as high-order consumer), water flea (as primary consumer), and green algae (as producer)—to assess the ecotoxicity of a pesticide in inland water ecosystems. "Inland water" covers extended areas from small streams to huge lakes, and there is also an immense variety of species. Taking into consideration the fact that susceptibility to pesticides differs among species, a predicted effect concentration is determined by dividing the toxicity values (EC_{50} and LC_{50}) in water flea and fish by a safety margin (= 10). To determine a more accurate safety margin, further ecotoxicity studies are needed using aquatic organisms which are important and representative of certain inland water ecosystems, in addition to the three species of test organisms.

In inland water ecosystems, the dominant primary consumer species are water flea and aquatic insects such as mayfly and caddisfly. Such ecosystems around paddy fields in Japan are characterized by numerous areas of running water, such as the rivers or irrigation canals that join the paddy fields. In many cases water fleas live in static waters, such as lakes. Running water is not their common habitat. In running water regions, the dominant species are mayfly and caddisfly. Because the mayfly has a complicated life cycle, it is considered very difficult to

breed. For this reason we selected the caddisfly instead as a target species. From among the caddisfly species we selected those of the family Hydropsychidae (especially *Cheumatopsyche brevilineata*), because they commonly live in the small rivers or irrigation canals directly connected to paddy fields throughout Japan. *C. brevilineata* was therefore suitable for evaluating the ecotoxicity of paddy pesticides. We developed an indoor breeding system to establish an acute toxicity study of caddisfly larvae.

The species of *C. brevilineata*, called as "Kogata-shima-tobikera" in Japan, inhabits gravel bottom of rivers or canals. The food of the larvae is mainly algae or organic matter flowing down the river. The larvae make silken thread nets, which they use to catch their food. Maturing larvae (fifth instar) make cocoons underwater. Hatched adults resemble moths, and the females lay egg masses on stony surfaces underwater. Under natural conditions the caddisfly has a bivoltine life cycle and overwinters in the larval stage (Fig. l).

We developed a system for indoor breeding of *C. brevilineata* (Fig. 2), using the following devices. First, we found that larvae could not survive with a shortage of oxygen in the water, so we maintained water flow in the

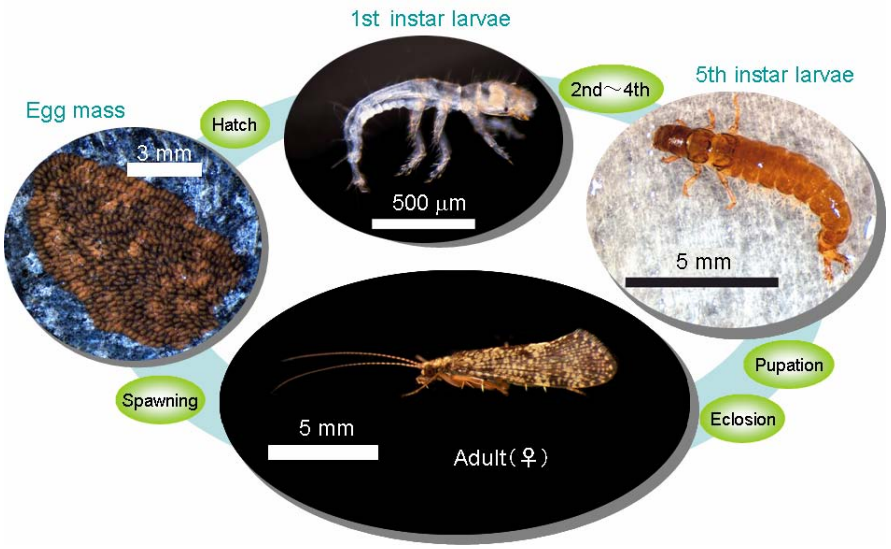

Fig.1 Life cycle of the caddisfly (*Cheumatopsyche brevilineata*).

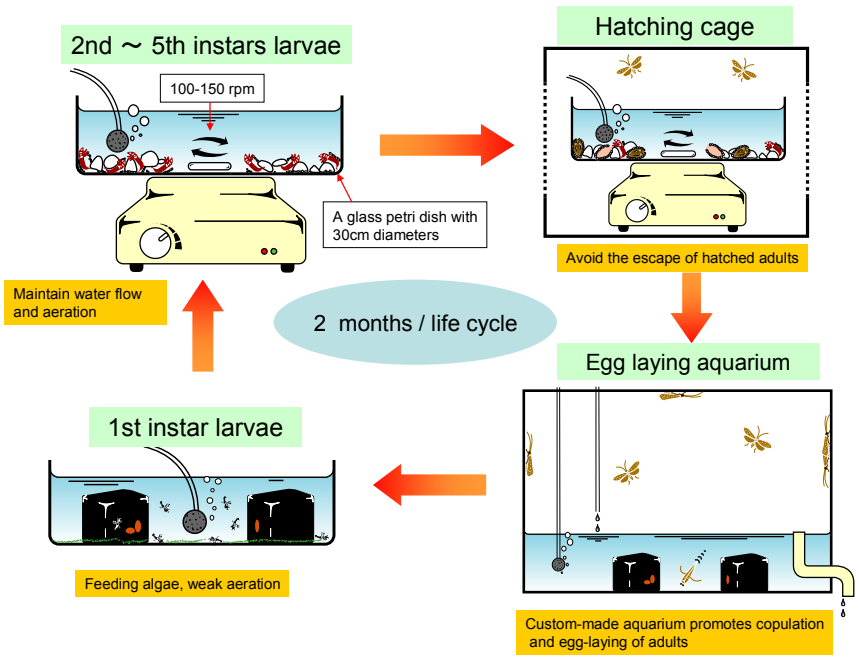

Fig.2 The indoor breeding systems of *C. brevilineata.*

vessel with a magnetic stirrer and aerated the water with an air pump. Second, when we fed only commercial flake fish food, the first instar larvae did not grow well and had a high mortality rate, so we improved their survival rate by feeding algae instead. Larvae at the second or greater instar were able to grow well when fed the commercial fish food. Third, we also devised a custom-made cage covering the breeding vessel to prevent the escape of hatched adults. Finally, we developed a custom-made aquarium to promote copulation and egg-laying of hatched adults. We filled the aquarium with water to a depth of about 8 cm and placed some cubic black rocks (about 5 cm square) in the water as sites for egg-laying.

We succeeded in breeding 4 or 5 generations a year of *C. brevilineata*, with 500 to 1000 individuals per generation, and were thus able to continuously supply first instar larvae for acute toxicity testing of pesticides. Further studies will be needed, but we expect to be able to develop chronic toxicity methods of *C. brevilineata* and apply our breeding system to other species of caddisfly. (K. Ohtsu and A. Yokoyama)

Topic 2: **Specific detection of strains of cotton aphid resistant to organophosphorus insecticides using nucleotide sequence differences in a detoxification enzyme gene**

Since the appearance of chemically synthetic insecticides in the 1940s, many examples of insecticide resistance in various species of agricultural and hygienic insect pests have been reported, and resistance has now been reported in over 500 species. The development and spread of insecticide resistance on arable lands have led to the application of excessive amounts of insecticides and have increased the negative impact on surrounding environments. Accurate and timely information on the status of resistant populations in the field is needed for mitigation of the risk associated with this resistance. Thus far, bioassays and/or measurement of detoxification enzyme activity have been the main methods used to determine resistance status. These traditional methods, however, are time consuming and costly and have high labor requirements, and the data obtained are of low accuracy. In addition, these methods cannot detect potential risky element carriers, namely insects heterozygous for recessive resistance genes or "revertants" whose resistance is regulated by methylation in the promoter region of the detoxification enzyme gene. Therefore, gene diagnosis would be the most effective way to accurately determine the status of, and trends in, the development of resistance.

The cotton aphid *Aphis gossypii* Glover is a serious pest of many crops and has developed resistance to various classes of insecticides. Overproduction of a detoxification enzyme, carboxylesterase (CE), had been proved to be responsible for OP insecticide resistance in *A. gossypii*. However, the nature of the differences in the CE between susceptible and resistant aphids has not been examined. We have been attempting to determine the CE-cDNA sequences in several strains of *A. gossypii*, with the aim of developing a gene diagnosis method for OP insecticide resistance.

We compared the CE-cDNA sequences in 1 super-susceptible (SS), 3 susceptible (S), and 4 resistant (R) strains reared in our laboratory. The LD_{50} values for fenitrothion, an OP insecticide, were 5.70, 22.4, or 113 ppm for the SS, S or R strains, respectively. Three different sequences were obtained, i.e. *ss*-, *s*-, and *r*-types (Fig. 3). Each sequence was divided into 2 regions: the amino acid coding region of CE, and the 5′ untranslated region forward of the CE region (Fig. 3). Prominent differences in length and nucleotide order were found in the later 5′ region sequences of these 3 types. SS strains had only the *ss*-type sequence, and S strains had only the *s*-type sequence. However, R strains had both *r*-type and *s*-type sequences. It was interesting to note that *r*-type cDNA was detected by real-time PCR approximately 20 times more frequently than the *s*-type. Therefore, R strains were distinguishable by the *r*-type sequence, and we consider that overexpression of *r*-type mRNA is responsible for resistance.

On the basis of these results, we developed a method

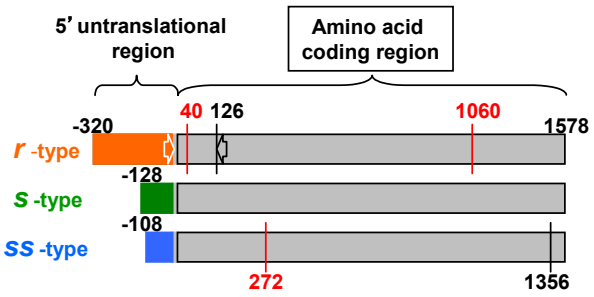

Fig.3 Comparison of the 3 CE-cDNA structures identified from organophosphorus-insecticide resistant, susceptible, and super-susceptible strains of the cotton aphid.

Vertical bars and numbers written on the amino acid coding regions of the *r*-type and *ss*-type indicate sites where nucleotide substitutions were found, in comparison with the *s*-type sequence. (Red and black bars respectively indicate sites with and without amino acid substitutions.) Sequences of the 5' untranslated region without homology among the three types are shown in different colors.
Arrows drawn on the r-type cDNA indicate PCR primers for specific detection of the resistant strains (see Fig. 4).

for the specific qualitative detection of OP insecticide-resistant cotton aphids. We designed a pair of forward and reverse primers related to CE. The forward primer was designed to anneal selectively to the 5′ untranslated region sequence of the *r*-type sequence. The reverse primer was designed to anneal to the amino acid coding region of CE with the *r*-type sequence by putting an *r*-type specific nucleotide site at the 3′ terminus of the primer (Fig. 3). By PCR using this primer pair, we could selectively detect resistant strains (Fig. 4). Unlike real-time PCR, this method does not require expensive apparatus or difficult quantitative processes such as adjustment of template concentrations among all samples and/or preparation of standard genes. Furthermore, this primer pair is effective not only for cDNAs as templates but also for genomic DNAs (Fig. 4). It is extremely useful as a rapid, simple, and accurate way of monitoring the status of resistance development. We are now attempting to improve the method: our targets are to skip the process of cleanup of the PCR templates and to downsize to downsize the analytical method for identifying the resistance with one individual of cotton aphid.. (K. Suzuki)

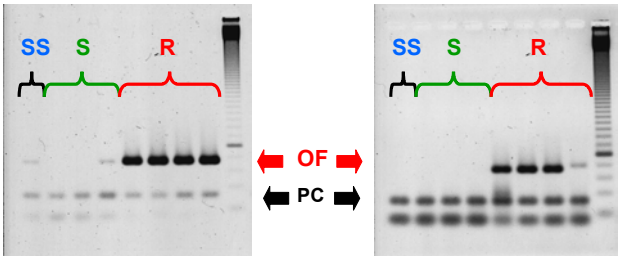

Fig. 4 Specific detection of organophosphorus insecticide- resistant strains of cotton aphid by PCR.

OF: Objective fragment
PC: Positive control (partial fragment of Acyl-CoA dehydrogenase)
R: resistant strains, S: susceptible strains,
SS: super-susceptible strain

2) Heavy Metal Research Group

The mission of the Heavy Metal Research Group is to elucidate the input–output balance of heavy metals such as cadmium (Cd) in arable soils and to clarify the mechanisms by which these metals are absorbed and translocated by paddy rice and soybean.

In FY 2004, the group studied the following 8 subjects: 1) phytoremediation of Cd-contaminated paddy fields by special rice varieties; 2) Cd input from rainfall in the city of Tsukuba; 3) remediation of Cd-contaminated paddy soil by washing with chemicals (see Main Research Results 1); 4) translocation characteristics of Cd absorbed by soybean (Topic 1); 5) effects of paddy field water management on the absorption of Cd by rice; 6) screening of soybean varieties for low Cd uptake and low accumulation in grains; 7) mechanisms of absorption and translocation of Cd in low-Cd-absorbing cultivars of rice (Topic 2) and soybean; and 8) evaluation of heavy metal loading of arable soils by fertilizers.

Topic 1: Uptake and transport of cadmium in hydroponically cultured soybean plants

If we are to effectively reduce the Cd content of seed, it is important that we determine the growth stages of the soybean (*Glycine max* var. Enrei) during which absorbed Cd is most likely to be transferred to the seed. The results of previous soil-pot and field experiments suggest that absorption of Cd before the beginning of the seed stage (R5) causes an increase in the Cd concentration of the seed. However, the details of this were still not clear. Using hydroponically grown soybean, we therefore evaluated the most critical stages of soybean development at which Cd absorbed via the roots was transferred into the seeds. Soybean plants at different growth stages were fed for 48 h with a culture solution containing Cd at 0.01 mg L^{-1}.

The Cd concentration in the seeds at the stage of full maturity (R8) differed depending on the growth stage during which the Cd was absorbed. The concentration of Cd in the seeds was highest when the soybean had absorbed Cd at the full pod stage (R4) through to the full seed stage (R6).

The cumulative Cd uptake by soybean seeds, as calculated from the rate of Cd uptake per day, is shown in Figure 1. Cd uptake during the full pod stage (R4)

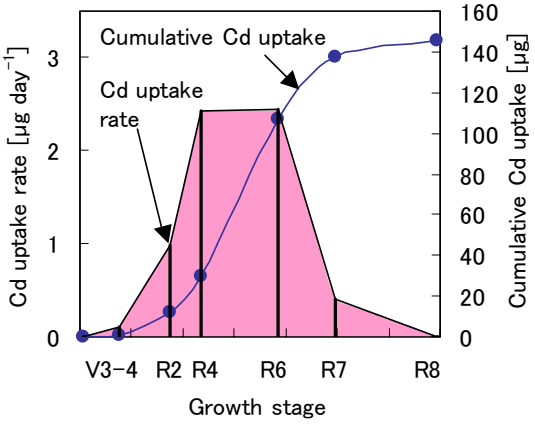

Fig. 1 Cadmium (Cd) uptake rate per day per plant and cumulative Cd uptake in soybean seeds per plant at 5 different growth stages.

through to the full seed stage (R6) accounted for approximately 50% (the largest proportion) of the total Cd content of the seeds. The amount of Cd absorbed during the third- or fourth-node stages (V3–V4) through to the full pod stage (R4) comprised approximately 20% (a minor contribution) of the total seed Cd content (Fig. 1). Therefore, to lower seed Cd content, it is important to reduce Cd absorption via the roots during the full pod stage (R4) through to the full seed stage (R6).

To investigate Cd transfer to the aerial parts, soybean plants to which we had applied Cd at the third- or fourth-node stage (V3–V4) were sampled at different growth stages. We then determined the Cd uptake in various aerial parts (Fig. 2). The absorbed Cd was transferred into the stems, petioles, and leaves until the full bloom stage (R2). At the full seed stage (R6) and the beginning of the maturity stage (R7), the Cd content of the leaves decreased steeply, whereas that in the pods and seeds increased. This result indicated that Cd accumulated in the leaves was translocated to the seeds during the full seed stage (R6) and the beginning of the maturity stage (R7).

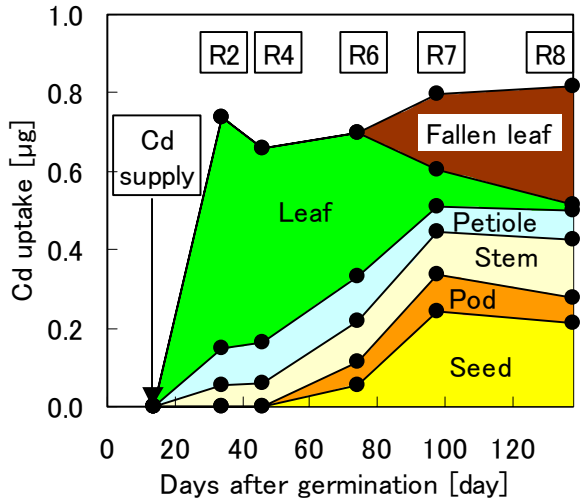

Fig. 2 Distribution of Cd in 6 parts of the soybean plant at 5 different growth stages. For 48 h each soybean plant at the third- or fourth-node stage (V3–V4) absorbed a culture solution containing Cd at 0.01 mg L^{-1}.

These results confirmed the tendency found in field experiments of Cd accumulation by soybean. To reduce Cd concentrations in soybean seeds it is important to develop practical soil management strategies to reduce Cd absorption during the R4 to R6 stages. (A. Kawasaki and H. Oda)

Topic 2: **Locating gene loci related to cadmium concentration in brown rice**

Of the daily foods of the Japanese people, rice is responsible for half the Cd uptake through the diet, and so we need to reduce concentrations of Cd in rice to levels as low as possible. The development of cultivars of brown rice that have low Cd contents is considered to be an effective permanent technology for reducing the uptake of dietary Cd. However, there has never been any research aimed at locating the QTLs (quantitative trait loci) that control the accumulation of Cd in brown rice. We attempted to locate the gene loci associated with Cd accumulation in brown rice by using a novel mapping population consisting of 39 chromosome segment substitution lines (CSSLs) in rice. Our aim was to obtain genetic information useful in the efficient development of cultivars with low Cd concentrations.

The 39 mapping populations, which carried a single chromosome segment of 'Kasalath' (*indica*) in each line overlapping with neighboring segments in a 'Koshihikari' (*japonica*) genetic background, were grown in pots (1/5000 a, four pots per CSSL) filled with Cd-contaminated soil (Gray Lowland soil, 0.1 mol L^{-1} HCl-extracted Cd concentration, 1.8 mg kg^{-1}). To elucidate the differences between 'Koshihikari' and each CSSL in terms of brown rice Cd concentration, we used growing conditions in which Cd was easily absorbed into the plant (water levels were maintained at 60% of field capacity). After grain-ripening, we collected the brown rice and measured its Cd concentration. Differences in grain Cd concentration between each CSSL and the recurrent parent 'Koshihikari' were evaluated by Dunnett's pairwise multiple comparison *t*-test ($P < 0.1$).

The brown rice of 3 CSSLs in which chromosomes 3 (SL-207 and SL-208) and 8 (SL-224) were partly substituted showed significantly lower Cd concentrations than that of 'Koshihikari'. The average Cd concentration of SL-223 was similar to that of SL-224, although the probability level somewhat exceeded the threshold of 0.1. On the other hand, 3 CSSLs in which chromosome 6 was partly substituted had significantly higher grain Cd concentrations than 'Koshihikari' (Fig. 3).

We investigated the phenotypic correlation between the Cd concentrations in the brown rice of the CSSLs and 16 quantitative traits representing agronomic, physiological, and morphological characters, and we found that the brown rice Cd concentration was correlated with 7 of those traits.

On the basis of the graphical genotypes of CSSLs whose brown rice showed significant differences in Cd concentrations relative to that of 'Koshihikari', we mapped the putative QTLs related to grain Cd concentra-

tion on chromosomes 3, 6, and 8 (Fig. 4). The results suggested that these QTLs might be linked to those for the 7 traits that had phenotypic correlations, indicating a pleiotropic effect of the QTLs for grain Cd concentration.

The use of CSSLs with a 'Koshihikari' genetic background could markedly shorten the time needed to breed 'Koshihikari' whose Cd concentration of brown rice is low. Because a 'Kasalath' segment on chromosome 6 carries a QTL related to a high grain Cd trait, it is important not to take in this region when using the effective genes on chromosome 6 in breeding. (S. Ishikawa)

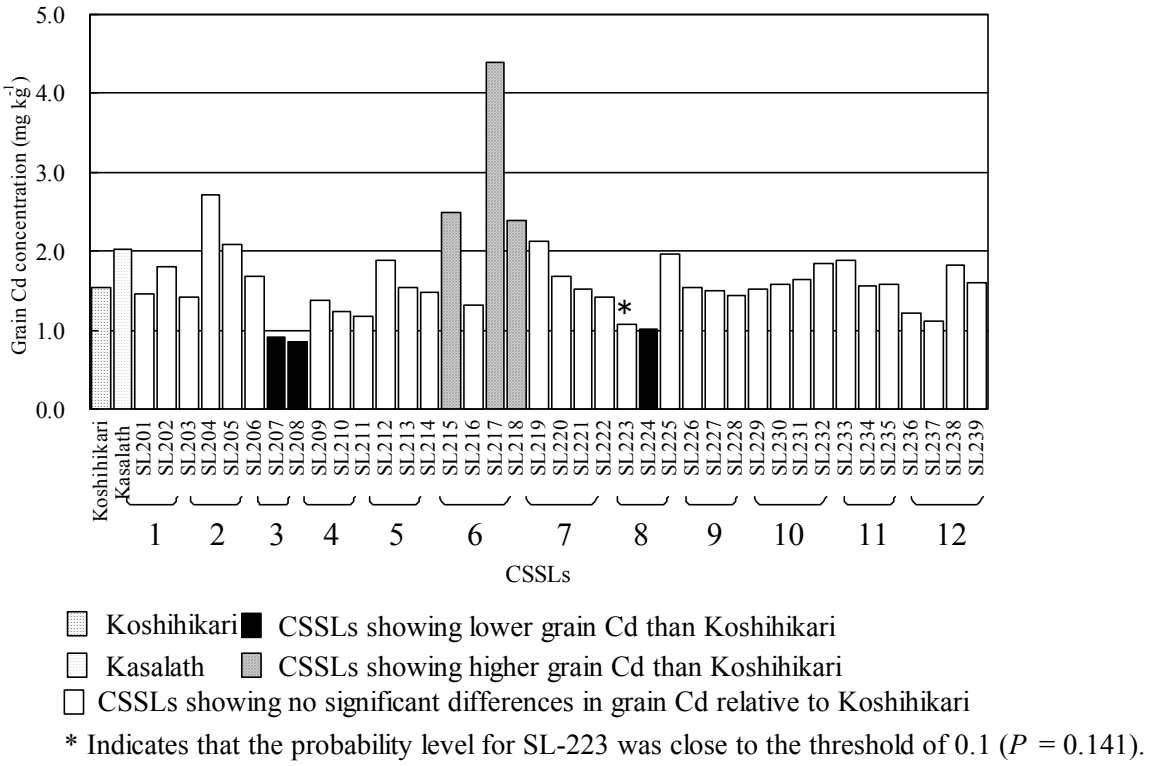

Fig. 3 Grain Cd concentration of CSSLs and their parents. The number under each name indicates the number of the chromosome at which each line was substituted.

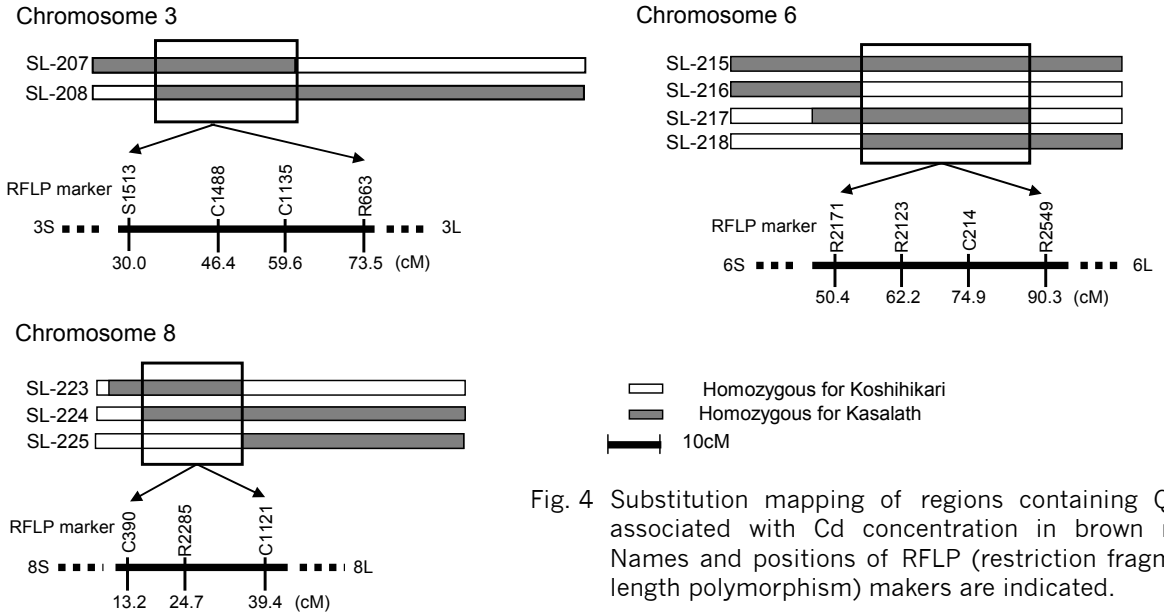

Fig. 4 Substitution mapping of regions containing QTLs associated with Cd concentration in brown rice. Names and positions of RFLP (restriction fragment length polymorphism) makers are indicated.

3) Water Quality and Solute Dynamics Group

The mission of the Water Quality and Solute Dynamics Group is to clarify the dynamics of solutes such as nitrate-nitrogen (NO_3-N) passing through arable lands to water bodies; to develop technologies to monitor loadings of NO_3-N and other pollutants; and to reduce these loads on the environment. We have 4 ongoing projects: 1) elucidation of the mechanisms of solute movement through the soil and below ground; 2) development of monitoring methods for NO_3-N and other pollutants in medium-sized river basins; 3) development of a technology for alleviating the agricultural nitrogen load on the environment by enhancing denitrification; and 4) construction of a model adaptable to medium-sized river basins for prediction of nitrogen load and effluent. In FY 2004, we elucidated the discharge of suspended matter and associated phosphorus to tile drains in a clayey field with subsurface cracks (Topic 1) and also estimated the origin of nitrous oxide in shallow groundwater under upland fields (Topic 2).

Topic 1: **Discharge of suspended matter and associated phosphorus to tile drains in a clayey field with subsurface cracks**

In general, phosphorus is adsorbed and precipitated strongly on soil particles and is insoluble in water, so that it hardly migrates vertically in soil. However, in fields with a clayey subsoil with shrinkage cracks—particularly ex-paddy fields that were used for paddy rice for many years and then recently converted for upland crops—it is likely that soil particles on which phosphorus is immobilized will migrate vertically through the cracks. To elucidate the process of discharge of suspended matter and associated phosphorus to tile drains in a clayey ex-paddy field, we measured the soil water condition, surface runoff, and tile discharge. We also collected water in the surface runoff and the tile drainage to determine the suspended particle (> 0.1 μm) and phosphorus concentrations.

In Niigata Prefecture we set up an experimental field on a Mottled Gley Lowland soil where soybean had been grown on an ex-rice paddy for 9 years and shrinkage cracks had opened in the clayey subsoil. Discharge of water to the tile drains was initiated soon after the pressure potential at the topsoil–plowsole boundary became positive (Fig. 1). The discharge responded quickly to changes in rainfall intensity, and there was no change in the water condition of the subsoil, suggesting that water flow occurred preferentially through the cracks (Fig. 1).

The concentrations of phosphorus in the tile drainage were highest at the beginning of discharge, with a maximum concentration of 1.3 mg-P/L. In tile drainage that had a concentration of phosphorus greater than 0.2 mg-P/L, more than 80% of the phosphorus was in the suspended form. During the period of soybean planting in 2004, about 90% (0.51 kg-P ha^{-1}) of the total discharge of phosphorus (0.57 kg-P ha^{-1}, about 5% of fertilization) to the tile drains was estimated to be in the suspended form. These values exceeded those for the suspended form of phosphorus (0.21 kg-P ha^{-1}) in surface runoff (0.27 kg-P ha^{-1}, about 2% of fertilization) (Fig. 2). These results demonstrate that subsoil cracks can be major pathways for the discharge of suspended soil particles and associated phosphorus from clayey ex-paddy fields. (K. Suzuki)

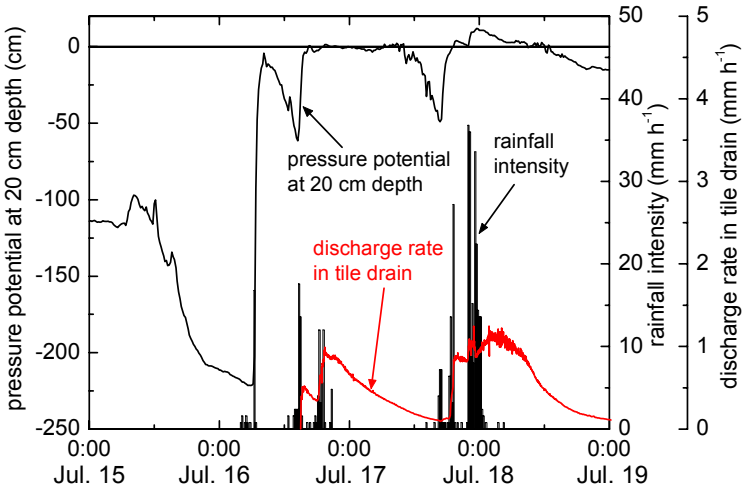

Fig. 1 Rainfall intensity, discharge rate to tile drains, and soil water potential at the topsoil–plowsole boundary during a rainfall event in mid-July in mid-July 2004.

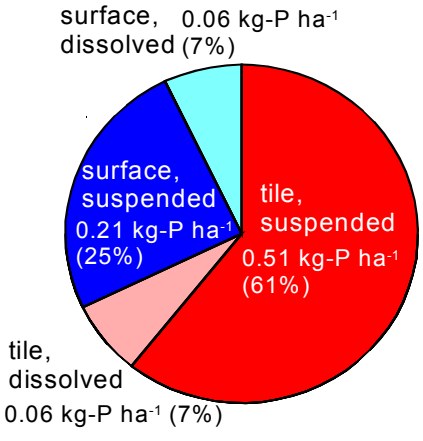

Fig. 2 Estimated discharge of phosphorus from an ex-paddy field during the monitoring period.

Topic 2: **Origin of nitrous oxide in shallow groundwaters under upland fields**

The nitrogen that originates from chemical fertilizers and animal wastes leaches mostly in the NO_3-N form and reaches the shallow groundwater. There, part of the NO_3-N is reduced by denitrifiers to N_2 gas or nitrous oxide (N_2O), which is an intermediate denitrification process. N_2O is often detected in spring waters. However, the denitrification activity and nitrogen dynamics in the groundwater remain to be determined. During a 3-year period of monitoring the quality of the groundwater that flows under upland fields in Ibaraki Prefecture, a high concentration of N_2O (20 to 5630 μg N/mL) was measured in a well. The soil (Cumulic Andosol) had usually received chemical fertilizers annually, and farmers had disposed of livestock excreta in trenches nearby until 1999.

In study well 2, the NO_3-N concentration was high when the water level was high, whereas the NH_4-N concentration was high when the water level was low (Fig. 3a). This suggests that NO_3-N originated from N applied as fertilizer, and the NH_4-N originated from manure. In the vicinity of the well, nitrogen seemed to have originated from different sources such as chemical fertilizers (lower $\delta^{15}N$) and livestock excreta (higher $\delta^{15}N$), which may have mixed together through leaching and horizontal migration. This assumption was supported by the finding that $\delta^{15}N$-TN, which did not include $\delta^{15}N$-N_2O, was low when the water level was high and high when the water level was low (Fig. 3b).

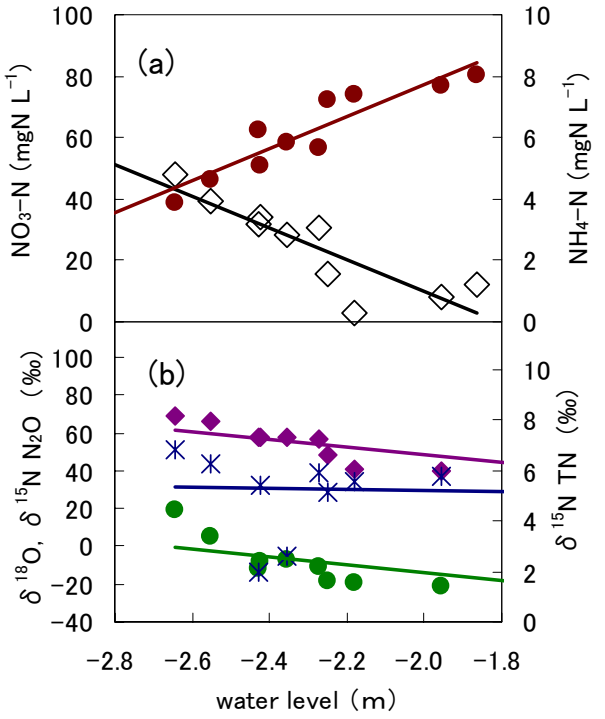

Fig. 3 Relationship between water level and NH_4-(◇) and NO_3- (●) N concentrations, $\delta^{18}O$ (◆), $\delta^{15}N$ of N_2O (●), and $\delta^{15}N$ of TN (＊).

In contrast, $\delta^{15}N$-N_2O was about –20‰ at high-water level and lower than $\delta^{15}N$-TN (Fig. 3b). This suggests that N_2O was produced by nitrification. The ratio of $\delta^{15}N$ to $\delta^{18}O$ of N_2O in the well was about 1 to 1; this ratio was far from 1 to 2, which is the usual value produced by the denitrification process. These results demonstrate that changes in $\delta^{15}N$ and $\delta^{18}O$ do not depend on denitrification in the groundwater, but can be produced by the mixing of "light" N_2O originating from fertilizer with "heavy" N_2O originating from manure. (Y. Nakajima)

4) Dioxin Dynamics Team

The mission of the Dioxin Dynamics Team is to elucidate dioxin dynamics in the agro-environment and to develop technologies to remediate dioxin-contaminated soils. In FY 2004 research was conducted on the following topics: 1) dioxin contamination patterns in crops; 2) temporal changes in concentrations of dioxins accumulated in Japanese paddy soils as a key to pollutant sources (Topic 1); 3) accumulation and behavior of dioxins in arable lands of Japan and Korea (Topic 2); and 4) development of a technology for reducing dioxin outflows from paddy fields.

Topic 1: **Temporal changes in organochlorine pesticide accumulation in Japanese agricultural soils**

Persistent organic pollutants (POPs) are transferred across borders and are accumulated in animals such as polar bears and seals. To prevent or reduce global environmental contamination caused by these compounds through cooperation with countries around the world, the Stockholm Convention on Persistent Organic Pollutants came into effect in May 2004. After the Convention is put into effect, countries will need to use its principles to manage the problems associated with POPs, by such means as prohibition of use, reduction of emissions to the environment, and prediction of future changes in contamination levels. Furthermore, most of the compounds specified as POPs in this agreement are organochlorine pesticides, such as DDT, dieldrin, and chlordane. It is, therefore, important that we understand the temporal trends in organochlorine pesticide concentrations in paddy soils so that we can predict future contamination levels. Paddy soils collected periodically from all over Japan since 1960 are preserved at NIAES. We analyzed these preserved soils to trace changes in organochlorine pesticide concentrations and to elucidate the sources of these contaminants.

The temporal change in organochlorine pesticide

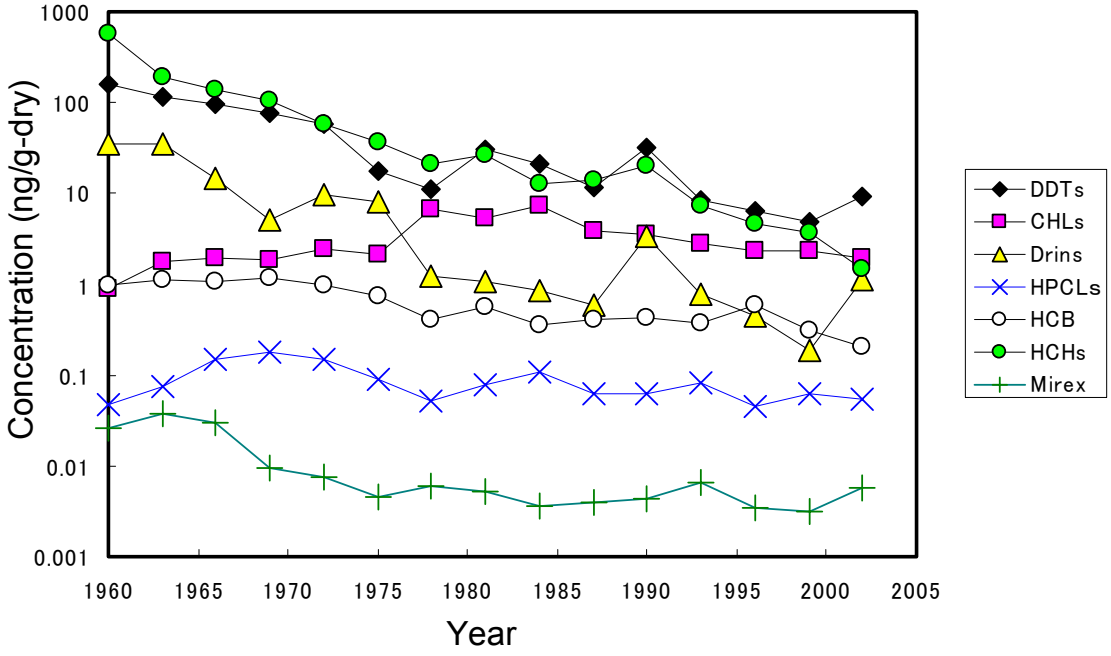

Fig. 1 Temporal change in organochlorine pesticide concentrations (ng/g·dry) in 15 paddy soils from Japan.

concentrations in paddy soils reflected the use of pesticides (Fig. 1). DDT, dieldrin, and HCH were used as insecticides on paddy rice, and their use was banned in the early 1970s; concentrations of DDT, dieldrin, and HCH in paddy soils have decreased drastically from the late the 1960s onward. Chlordane was used until 1986 to control domestic pest insects, but was not used in paddy fields in Japan; concentrations of chlordane in paddy soils increased from the 1970s through to the 1980s, suggesting that chlordane was transported via the atmosphere and/or water during the period when it was used. Concentrations have since been gradually decreasing. Despite the fact that hexachlorobenzene (HCB) was never used as a pesticide in Japan, we detected it in paddy soils throughout the entire period studied. One of the important sources of HCB is impurities of pentachlorophenol (PCP), which was used in large quantities as a paddy herbicide during the 1960s in Japan. Concentrations of HCB in paddy soils increased during the 1960s but have been gradually decreasing since; presumably HCB had accumulated in paddy soils as an impurity in PCP herbicides. Mirex, an insecticide, was never used in Japan, but a small amount of mirex was detected in paddy soils during the entire period, suggesting that mirex was transferred across borders from other countries. (N. Seike)

***Topic 2:* Annual mass balance of dioxins in a Japanese paddy field**

1. Dioxins in Japanese paddy soils

According to the Ministry of the Environment (MOE), the mean dioxin concentration in Japanese paddy soils in 2001 was 46.5 pg-TEQ (toxic equivalent)/g, a value higher than those in upland soils. The mean concentration in NIAES experimental paddy soils was 82 pg-TEQ/g, a little higher than average but within the range of normal paddy soils. The dioxins were derived from impurities in the herbicides chloronitrophen (CNP) and pentachlorophenol (PCP), which were used until they were banned in 1965. We therefore estimated the annual mass balance of dioxins in a Japanese paddy field to predict changes in their concentration in paddy soils. The following factors that influence the concentration of dioxins in paddy soils had to be taken into account: (1) factors that increase dioxin concentrations in soil: atmospheric deposition, input as rice plant parts, and input from irrigation; (2) factors that decrease dioxins in soil: runoff, decomposition, and volatilization. We estimated the values of these factors from our experimental data and references.

2. Factors that increase dioxin levels

Atmospheric deposition: Our data from the NIAES field experiments showed that the dioxin input from the atmosphere was 2.0 ng-TEQ m^{-2} $year^{-1}$.

Input as rice plant parts: The mean dioxin concentration in the leaves and stems of rice was 4.3 pg-TEQ/g (our experimental data). Supposing that the yield was 700 g/m^2, the input to the paddy soils in the form of rice leaves and stems would have been 3.0 ng-TEQ m^{-2} $year^{-1}$.

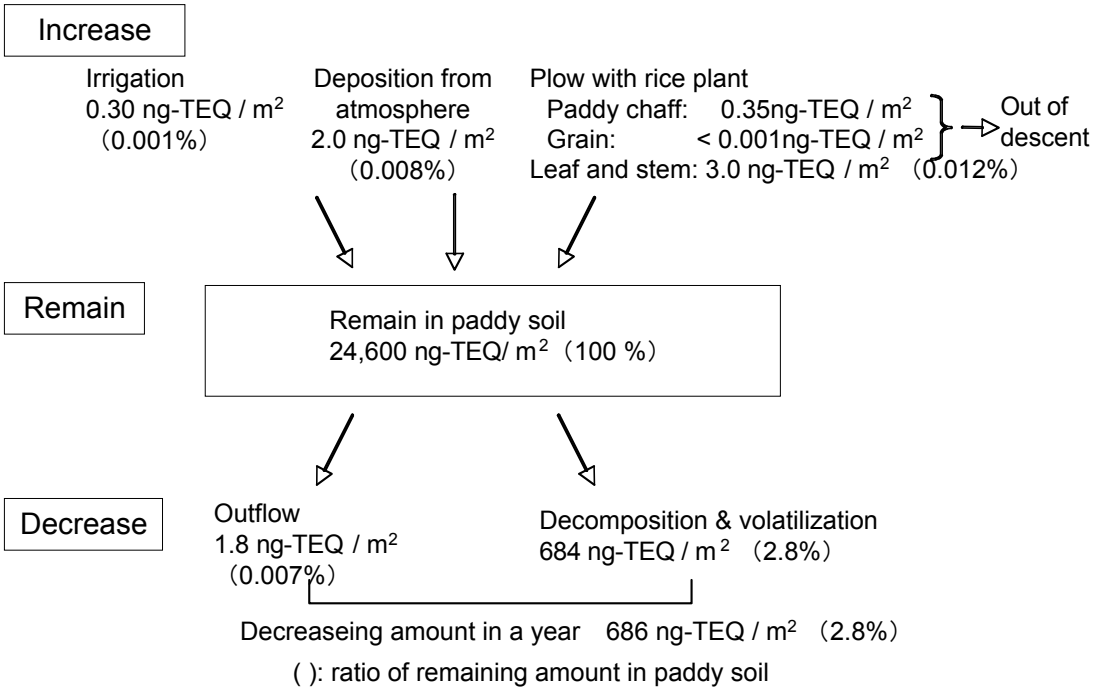

Fig. 2 Annual balance of dioxins in a Japanese paddy soil

Input from irrigation: The average concentration in irrigation water was 0.25 pg-TEQ/L (environmental assessment data from MOE). The dioxin input from irrigation to paddy soils was estimated to be 0.30 ng-TEQ/m^2.

Dioxin concentrations in agricultural chemicals, including fertilizers, were negligible. Therefore, the total increase in dioxin levels was calculated to be 5.3 ng-TEQ/m^2.

3. Factors that decrease dioxin levels

It is impossible to measure the decomposition and volatilization of dioxins in soils. Therefore, we tried to estimate this loss from the half-lives of these chemicals remaining in the soil. The mean amount of dioxins remaining in the NIAES paddy soils was 24 600 ng-TEQ/m^2. MAFF estimates the reduction half-term to be 25 years. From this estimate, we calculated a disappearance rate constant (K) equal to 0.0277 (/year). As a result, the annual decrease in dioxin levels in the NIAES paddy soil was calculated to be 681 ng-TEQ/m^2. This net decrease in dioxin concentration is the balance between the input and the output, including decomposition. The gross decrease is composed of runoff from the field, decomposition, and volatilization. Because our experimental data showed that the mean runoff of dioxins through drainage during flooding was 1.8 ng-TEQ/m^2, the decrease in dioxins due to decomposition and volatilization was 684 ng-TEQ/m^2.

4. Annual mass balance of dioxins in a Japanese paddy field

The results of these calculations are summarized in Figure 2, with the ratio of each amount to the total remaining in the soil shown in parentheses. Compared with the amount remaining in the soil, the amounts of increase and decrease in dioxins are very small, indicating that there has been little change, or only a very slow decrease, in dioxin concentrations in paddy soils. (R. Uegaki, N. Seike, and T. Otani)

Natural Resources Inventory Center

The mission of the Natural Resources Inventory Center is: 1) to perform fundamental research on the classification, identification, characteristics, and functions of agro-environmental resources such as soils, insects, and microorganisms; 2) to promote and support research through the collection, preservation, exhibition, lending, and donation of specimens and samples; 3) to store agricultural environment information in databases and develop inventory systems that can be accessed with the aid of tools such as the Internet; and 4) together with related research groups, to collect and evaluate microbial and insect genetic resources as a sub-bank under the MAFF GeneBank Project. The Natural Resources Inventory Center has 3 laboratories: Soil Classification, including the Soil Museum; Insect Systematics, including the Insect Museum; and Microbial Systematics, including the Microbe Museum. As part of the mid-term research plan of NIAES formulated in FY 2001, these laboratories have carried out the following research: 1) classification and elucidation of the functions of soils and development of a framework for the soil inventory; 2) construction of a database for type specimens of insects and development of a framework for the insect inventory; 3) classification and identification of microorganisms co-inhabiting sound plants, analysis of their functions, and development of a framework for the microorganism inventory; and 4) collection and evaluation of insects and microorganisms as genetic resources under the MAFF GeneBank Project.

Major topics in 2004–2005 are described in the following Topics and in "Invasion and occurrence of the banana moth, *Opogona sacchari* (Bojer) (Insecta, Lepidoptera), over a wide area of Japan", in the Highlights on page 9.

Topic 1: **Newly opened Natural Resources Inventory Museum**

"Inventory" means a list of property or stock, and our research institute uses this word in the sense of a bank storing specimens and information on agricultural environmental resources. For visitors' convenience in understanding the purpose and content of agricultural environmental inventory research, part of the Soil Preservation and Monolith Experiment Building has been refurbished as the Insect and Microorganism Exhibition Room, and a Natural Resources Inventory Museum has been opened. The entrance hall has exhibits of typical soil monoliths (soil profile specimens) from the hilly to the coastal regions of our country, as well as displays on such subjects as how soil monoliths are created. On the left-hand side of the entrance hall are located the Soil Sample Storage Room and the Soil Monolith Exhibition Room, where soil samples have been stored for 50 years. The Insect and Microorganism Exhibition Room is located to the right-hand side of the entrance hall. The exhibition theme there is "Diverse Insects and Microorganisms in the Agricultural Environment". In this room can be seen the results of research on the insects and microorganisms that inhabit the agricultural environment, as well as various specimens. Furthermore, by reflection on a large-sized liquid crystal display, many visitors can simultaneously search the various databases released by our research institute. (Y. Ueda)

Topic 2: **Soil resource inventory for classification**

We surveyed subsoils to 5 m depth in the Omoi River area and analyzed their physico-chemical properties. Data on the physical and chemical properties of subsoils are essential for soil classification and evaluation of nitrate-carrying capacity in arable land. We compiled databases on soil monolith information and on the results of soil surveys and research projects on heavy metals, and we developed a browser for the soil monolith database. We devised a soil information system by developing the functions of input, search, edit, and display of soil maps and statistical data on the Internet. We also compiled a data set of soil profiles based on the results of soil surveys of arable lands in Japan; this data set is available for use with the EPIC model. We modified the EPIC model program by using C++ program language under the Windows OS to adapt it to Japanese soils. The average, median, standard deviation, mode, and maximum and minimum values of Cd, Cu, Zn, and Pb contents in Japanese arable soils were calculated from our heavy metal database. Soil genesis and heavy metal content were investigated on 2 series of river terraces by using principal component analysis. Surface analysis of soil thin sections by electron probe microanalysis (EPMA) showed heavy metals accumulated around areas of mottling, together with iron. We prepared a manual for estimating and mapping Cd risks in soybean from prediction equations derived from the relationship between Cd content in soybean and soil properties. (M. Nakai)

Topic 3: **Bacteria and fungi on healthy leaf sheaths and panicles of intact rice plants**

To study the microbial communities on rice plants, we isolated bacteria and fungi from the leaf sheaths and panicles of intact rice plants cultivated in paddy fields in Tsukuba, Japan, between 2001 and 2003, Two leaf

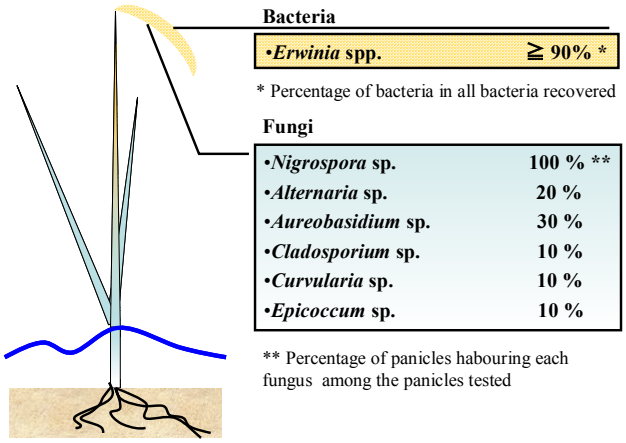

Fig. 1 Dominant bacteria and fungi on rice panicle

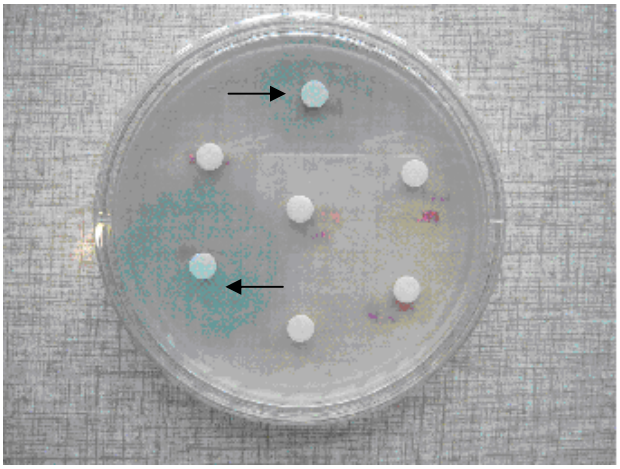

Fig. 2 Production of full QSRM on detection medium by bacteria isolated from rice plants.
A culture filtrate of the bacteria was placed onto a round filter paper. Arrows indicate the QSRM, which are detected as a diffuse blue pigmentation.

sheaths per hill were sampled; one was used for isolating bacteria and the other for fungi. Ten sheaths were used for each experiment. The sample was washed in a washing buffer (10 mM phosphate buffer, pH 7.0; WB) on a shaker to isolate epiphytic bacteria. Before being washed, the sample was covered at both ends (ca. 5 mm) with melted paraffin to avoid contamination with microorganisms from inside the leaf sheaths. Appropriate dilutions of the buffer solution containing the isolates were plated onto nutrient agar (NA). The washed samples were ground in a mortar by adding WB for further isolation of the bacteria remaining in or on the leaf sheath, and the dilutions were plated onto NA. Bacterial population sizes were estimated after 5 days of incubation at 25 °C, and 25 colonies per sample were selected for the analysis of 16S rDNA sequences. For isolation of fungi, each panicle was washed in WB, cut into 5 subsamples (1 cm long), and then incubated on water agar plates for 28 days at 23 °C. The bacterial populations ranged from 10^4 to 10^5 and 10^5 to 10^7 cfu/g fresh weight, respectively, in the washing and ground solutions of the leaf sheath, respectively. Bacteria belonging to the genera *Microbacterium* and *Sphingomonas*, and a few different fungi (*Cladosporium*, *Alternaria*, and *Epicoccum*) were most frequently isolated 1 month before heading. The predominant microflora at heading time consisted of *Sphingomonas* (bacterium) and *Cladosporium*, *Phaeosphaeria*, and *Nigrospora* (fungi). Two panicles (ca. 5 cm long) per hill were sampled at heading time by cutting with sterilized scissors. One sample was used for isolation of fungi and the other for bacteria. Bacteria belonging to the genus *Erwinia* were most frequently isolated from the panicles at heading time. Among the fungi, *Nigrospora*, *Aureobasidium*, and *Alternaria* were predominant (Fig. 1).

We also analyzed the production of cell–cell communication-related signal molecules (quorum sensing-related signal molecules, QSRM) in the bacteria isolated from rice plants (Fig. 2). (S. Tsushima)

Chemical Analysis Research Center

The Chemical Analysis Research Center has been developing new methods of analyzing environmental chemicals and has been studying the fate of these compounds in plants, water, and soils.

The **Environmental Chemicals Analysis Laboratory** has been studying the fate of organoarsenics such as diphenylarsinic and phenylarsonic acids in paddy fields. By using an LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry) system we have developed a new method for analyzing cadmium (Cd) concentrations in grains such as rice, wheat, and soybean. We are able to measure the Cd concentration in grains without the need for pretreatment, and we have clarified the distribution of Cd in rice grains. Furthermore, our laboratory has developed a convenient method of clean-up samples for dioxin analysis. Gel permeation chromatography is the most effective way to remove the waxes from plant samples destined for dioxin analysis (Topic 1). Laboratory members are also studying the use of ELISA (enzyme-linked immunosorbent assay) for the detection of pesticide residues in crops. In September 2004 we ran a training course on "How to measure pesticide residues in crops by ELISA".

The **Radioisotope Analysis Laboratory** has been surveying the fallout of artificial radioisotopes such as ^{137}Cs and ^{90}Sr in wheat, rice, and soils collected from all over Japan since 1957. These data have been used to determine the fate of these radioisotopes in the soil. Laboratory members are also studying prediction of the fate of ^{129}I discharged from nuclear fuel processing plants, and they have constructed a system for the large-scale extraction of iodides from soil and plants. The results suggest that 0.1 Bq ^{129}I / kg soil may be detected. To clarify the fate of iodides in paddy fields, we have been using the XANES (X-ray absorption near edge structure) method to investigate changes in the chemical form of iodides in the soil (Topic 2).

Yellow sand frequently blows across to northern Japan in the early spring. Because the fallout of artificial radioisotopes such as ^{137}Cs and ^{90}Sr has also been increasing in these areas, we have begun a study to determine the source of the yellow sand.

Dr. Noriko Yamaguchi, researcher at the Radioisotope Analysis Laboratory, was awarded a progress award by the Japanese Society of Soil Science and Plant Nutrition.

Topic 1: **Clean-up of species interfering with dioxin analysis in leafy vegetables**

In general, the analysis of PCDD/Fs and PCBs requires labor-intensive, multi-step clean-up procedures that are both expensive and time consuming. Therefore, a rapid and reliable analytical method is needed. Recently, we reported on the efficiencies of PCDD/Fs analyses based on a variety of extraction techniques such as supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and automated soxhlet techniques. In Japan, dioxin analysis in foods is essentially performed according to the "Provisional guidelines for methods of analysis of dioxins and Co-PCBs in food" (Ministry of Health, Japan, 1999). However, because vegetables contain various interfering species, such as oils, pigments, or waxes, an additional clean-up procedure is required high clean-up efficiencies for the dioxins analysis. To remove interfering species (long-chain aliphatic compounds) from leafy vegetables, we tested the application of conveniently available clean-up procedures, using a homemade multi-layer silica gel column combined with a Carboxen 1016 (75 mg) short column (Fig. 1). Figure 2 shows the interfering species in the mono-*ortho* PCB fraction after a typical column chromatography clean-up of leafy vegetable samples on the basis of the present Japanese dioxin analysis method. The results obtained by ^{1}H, ^{13}C NMR and GC–MS indicated that these species were mainly hentriacontane ($C_{31}H_{64}$), accompanying nonacosane ($C_{29}H_{60}$), components of the pathway for synthesis of the principal components of wax. Elution with hexane revealed that the major fractionation of mono-*ortho* PCBs occurred in the 30 to 80 mL hexane eluate. Thus (Fig. 3), the 0 to 30 mL hexane elution step

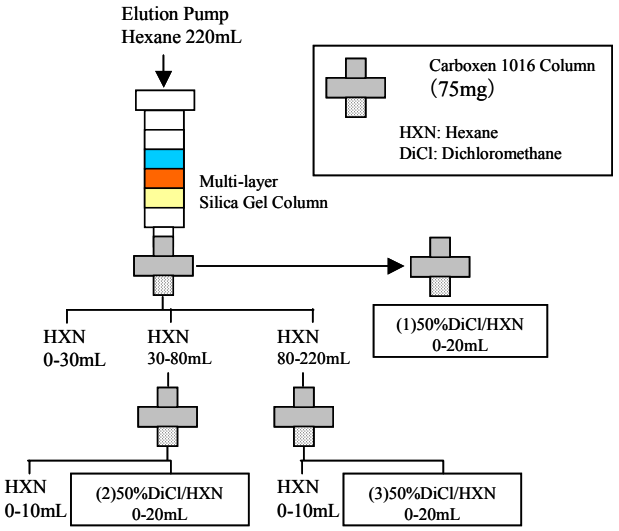

Fig. 1 Procedure used for clean-up of a mono-*ortho* PCBs fraction.

allowed us to remove hentriacontane and nonacosane from spinach samples. The interfering species (alkanes) were found mainly in the hexane eluate. We confirmed that hentriacontane and nonacosane could be removed by washing with hexane using a Carboxen 1016 short column.

Thus, almost nothing of the interfering species (alkanes) remained in the 50-vol% dichloromethane/hexane elution (Fig. 1 (1), (2), (3)). The sum of the recovery percentages of mono-*ortho*-Co-PCBs in the spinach samples was over 80% (Fig. 4). We concluded that the proposed method of using a Carboxen 1016 short column is effective on removing long-chain alkanes from leafy vegetables. (H. Eun)

Topic 2: **Cause of eluviation of iodine in flooded paddy fields**

Because of its very long half-life (16 million years), iodine-129 is one of the most hazardous gaseous radionuclides leaked from nuclear fuel reprocessing plants. For the sake of food safety and security, it is critical that the behavior of ^{129}I in agricultural environments be investigated. A previous study in our laboratory revealed that iodine concentrations in paddy field soils were substantially lower than in upland fields and forest soils. The drop in redox potential during the period when the paddy soils are flooded may cause the reduction of IO_3^- to I^-, and, as a result, the solubility of iodine may increase. By applying nondestructive analysis using XANES (proposal no. 2004B0093-NXa-np), we investigated whether changes in the state of oxidation of iodine species occur

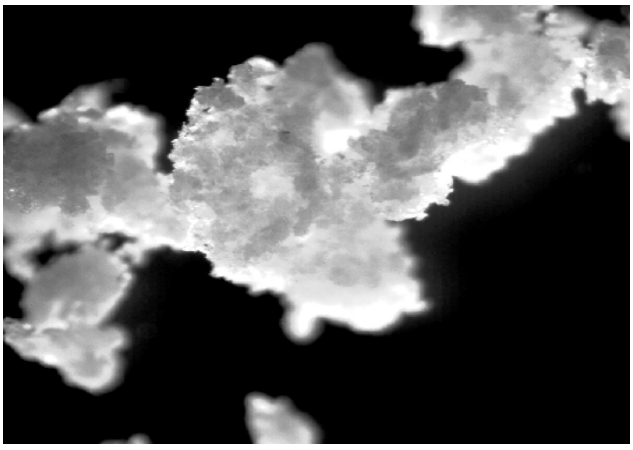

Fig. 2 Crystals of interfering compounds obtained during the clean-up procedure.

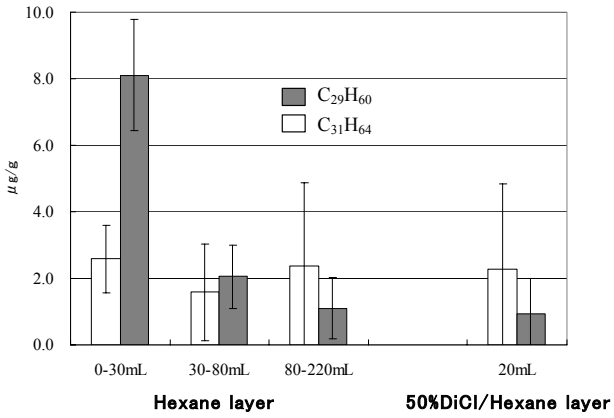

Fig. 3 Concentrations of hentriacontane ($C_{31}H_{64}$) and nonacosane ($C_{29}H_{60}$) in each fraction.

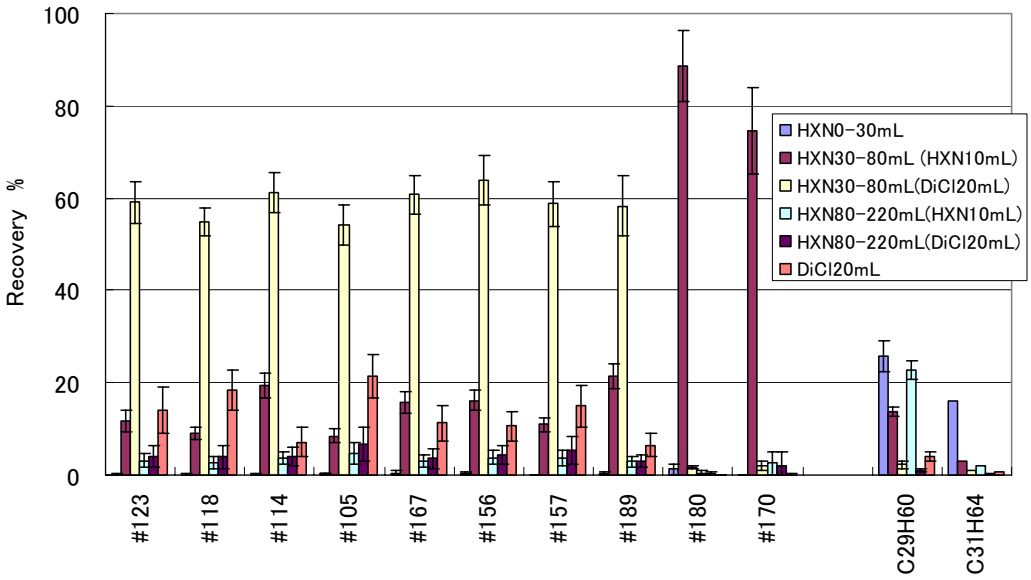

Fig. 4 Percentage recovery of mono-*ortho* PCBs in each fraction from spinach samples.

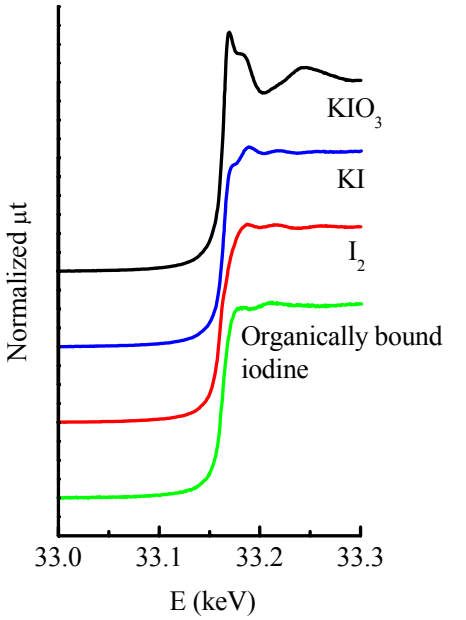

Fig. 5 XANES spectra of iodine reference materials.

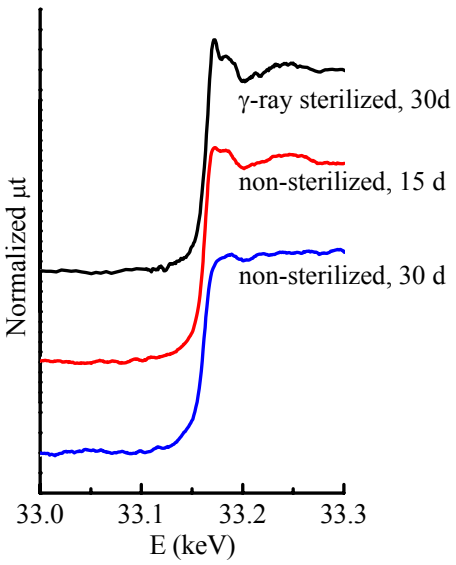

Fig. 6 XANES spectra of iodine on paddy soil after incubation under flooded conditions.

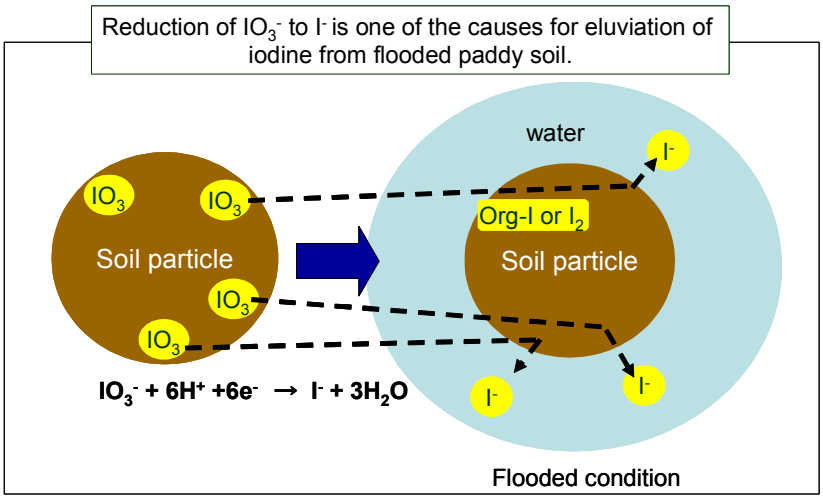

Fig. 7 Mechanism of iodine eluviation in a flooded paddy soil.

in flooded soil systems and thereby affect the species' mobility in the soil environment.

Figure 5 shows the reference XANES spectra of KIO_3, KI, I_2, and organically bound iodine. Iodate (IO_3^-) had a very obvious post-edge structure, whereas those of KI, I_2, and organically bound iodine were weak. The XANES post-edge feature distinctively indicated the presence of IO_3^- in the sample. Figure 6 shows the XANES spectra of iodine that was spiked into paddy soils as IO_3^-, after which the soils were incubated for 15 or 30 days. Disappearance of the XANES post-edge feature of IO_3^- showed that the decreased concentration of iodine in the paddy soil was the result of a reductive reaction of IO_3^- when the paddy field was under anaerobic conditions. The reduced products would be I^- and I_2 or organically bound iodine. The concentration of I^- in solution phase increased after incubation under flooded conditions. Therefore, the I^- formed was not retained by the soil but was dissolved into the solution phase, whereas I_2 or organically bound iodine remained in the solid phase of the soil. Sterilization of the paddy soils inhibited the reduction of IO_3^- (Fig. 6). In conclusion, biological consumption of oxygen in the soil and the subsequent drop in redox potential are important to the reduction reaction of IO_3^- and cause eluviation of iodine as I^- in paddy soil systems (Fig. 7). (N. Yamaguchi)

Research Project

1. Current and Future Status of Contamination of Japanese Agricultural Fields with POPs and Related Compounds

Persistent organic pollutants (POPs) are transferred across borders and are accumulated in animals such as polar bears and seals. This means that POPs are causing contamination on a global scale. To prevent or reduce global environmental contamination caused by these compounds through cooperation among countries around the world, the Stockholm Convention on Persistent Organic Pollutants was adopted in Stockholm in May 2001 and came into effect in May 2004. Most of the compounds specified as POPs in this agreement are organochlorine pesticides such as DDT, dieldrin, and chlordane. Because the Convention is now in effect countries will need to use its principles to manage various problems related to POPs, by means such as prohibition of use, reduction of emissions to the environment, and prediction of future changes in levels of contamination.

The purposes of this project are to elucidate the status of contamination in the agricultural fields with POPs and related compounds from the 1960s up to the present, and to predict future changes in contamination with these compounds, by developing a multimedia model that considers diffusion and outflow to the atmosphere and the aquatic environment from agricultural fields in Japan. This project is composed of the following three subjects: (1) Temporal changes in the status of organochlorine pesticide contamination of agricultural soils; (2) Temporal changes in the status of polycyclic aromatic hydrocarbon (PAH) contamination of agricultural soils; (3) Development of an environmental fate model for POPs and related compounds.

The project was started in FY 2004 and is scheduled to end in FY 2006. In the first year, we obtained some new findings with regard to subject (1): Temporal changes in organochlorine pesticide concentrations in paddy soils reflected the use of pesticides. DDT, dieldrin, and HCH were used on paddy rice until their use was banned in the early 1970s; concentrations of these pesticides have decreased dramatically since the end of the 1960s. Chlordane was used to control domestic pest insects until 1986 but was not used in paddy fields; concentrations of chlordane in paddy soils increased from the 1970s through the 1980s, but since then they have steadily decrease, suggesting that chlordane was transported via the atmosphere and/or water during the period when it was used. Mirex, an insecticide, was never used in Japan, but small amounts of mirex have been detected in paddy soils the 1960s up to the present. Therefore, mirex appears to have been transferred across borders from other countries.

2. Pilot Study in North-East Asia for the Development of Desertification Assessment and Construction of an Early Warning System

The development of benchmarks and indicators for the monitoring and assessment of desertification and the establishment of operational and cost-effective early warning systems (EWSs) for drought and desertification were among the principal items on the agenda drawn up by the Committee on Science and Technology under the United Nations Convention to Combat Desertification (UNCCD).

Desertification occurs through a complex combination of elements on a local scale. However, at the same time, the phenomenon manifests itself on a larger scale. Large-scale monitoring studies of desertification have not been able to gather concrete information on the desertification process at a local level. On the other hand, the results of studies at the local scale have not been connected to those of large-scale assessments of desertification. In other words, previous studies on desertification have not been able to fill the gap between local and large scales. This project employs an integrated desertification model to fill that gap and to develop cost-effective EWSs for desertification. Field-scale assessment of desertification and large-scale observations will be integrated by employing the model.

This project is being supported financially by the Global Environment Research Fund of the Ministry of Environment, Japan, from FY 2004 to 2006. It aims to 1) establish desertification EWSs by employing an integrated model; 2) standardize observation methods for long-term monitoring of desertification indicators on a large scale; and 3) assess land vulnerability through field surveys of soil and vegetation.

The outline of the project is as follows:

1) Construction of an integrated model for desertification EWSs
2) Standardization of observation methods for long-term monitoring of desertification indices
3) Land vulnerability assessment by soil/vegetation/hydrological analysis
   (1) Land vulnerability assessment by field survey
   (2) Physiological and ecological assessment of soil degradation

3. Development of a methodology for assessing risk of crop contamination with Cd on arable lands

Public concern about food safety has been increasing. The international organizations FAO and WHO have been examining a new international safety standard for Cd in foods to minimize the human intake of this heavy metal because it is a matter of urgency that we manage the risk of Cd in foods. In this light, this project, which ran from FY 2002 to FY 2004, was initiated to secure food safety and, in particular, to minimize Cd concentrations in crops. The aim of the project was to develop methods of assessing the risks of Cd contamination of edible parts of crops on arable soils and to reduce Cd uptake by crops from soils. Our main results are summarized below.

1) Method of determining amount of Cd in soil available to crops:

We used porous cups buried in the soil to collect soil solution from paddy fields during the growing season. We found that the degree of Cd contamination in rice could be estimated from the Cd concentration in the soil solution. For upland crops such as beans, wheat, and vegetables, the 0.01 N hydrochloric acid (HCl) extraction method was more suitable for predicting Cd contamination than conventional methods such as 0.1 N HCl extraction.

2) Analysis of soil physico-chemical properties in relation to crop Cd contamination:

Using the database of Cd contamination of crops and vegetables, we analyzed the relationship between crop Cd concentration and soil physico-chemical properties. Multi-variable analysis of the database showed that the degree of Cd contamination of crops could be predicted from 3 soil parameters: Cd concentration, pH, and phosphate absorption capacity. This prediction method was verified in various upland crops grown by farmers in the Hokkaido region and can be applied to the construction of risk maps for Cd contamination.

3) Balance of Cd in arable soils:

We monitored the Cd loading of fields from irrigation and atmospheric dusts and the Cd concentrations in drainage and soil solutions during a growing season. Under common agricultural practices, the risk of Cd contamination may be almost negligible, even if this Cd loading of the soil continues for 100 years.

4) Differences in Cd uptake among cultivars of rice, soybean, and spinach:

Among rice cultivars, early maturing varieties showed a lower risk of Cd contamination of the grain than did late varieties. We also found that soybean cultivars could be divided into 2 distinct groups of lower and higher seed Cd concentration. Cultivars showing higher Cd concentrations could be identified from the Cd concentration in the shoots of the young plant. Spinach cultivars bred in foreign countries tended to show higher Cd concentrations than did Japanese cultivars. Soil amelioration by calcium amendment may reduce the contamination risk.

The outcomes of the project were communicated to MAFF policymakers and contributed to the development of a policy on systems of management for the reduction of Cd contamination risk.

4. A FACE (free-air CO_2 enrichment) study to predict the impacts of atmospheric CO_2 increase on agricultural ecosystems

The projected increase in atmospheric carbon dioxide concentration ($[CO_2]$) and climate change will have significant impacts on future agricultural productivity. To ensure food supplies under changing environments, we need to better predict the impacts and develop agricultural technologies that can adapt to these future conditions. We therefore undertook this project, in collaboration with the National Agricultural Research Center for the Tohoku Region, supported by the Global Environment Research Coordination System of the Ministry of the Environment, to predict the impacts of atmospheric CO_2 increase on rice production and agro-ecosystems. For this purpose, we conducted FACE (free-air CO_2 enrichment) experiments at Shizukuishi in Iwate Prefecture. We also performed TGC (temperature-gradient chamber) experiments and improved a crop growth model and a biogeochemical model on the basis of our experimental observations.

This FACE treatment simulated a $[CO_2]$ condition 200 ppmv above the ambient, with no enclosures in the paddy field, to minimize experimental artifacts. Comparison of biomass enhancement in response to elevated $[CO_2]$ amongst cultivars, nitrogen treatments, and years indicated that low plant N concentration at the panicle initiation stage resulted in low biomass enhancement at harvest. Although elevated $[CO_2]$ significantly reduced the degree of lodging under heavy N fertilization, susceptibilities to blast and sheath blight diseases and chilling-induced spikelet sterility were exacerbated by elevated $[CO_2]$: the mechanisms of these non-photosynthetic effects on grain yield need further investigation.

A generic crop growth model (MACROS, Modules of an Annual CROp Simulator), which was developed to express plant carbon metabolism, greatly overestimated the effect of elevated $[CO_2]$ and underestimated the effect of N application. We therefore developed a model

that introduced Farquhar's photosynthesis model and N-driven leaf area dynamics and was able to reflect the interactive effects of [CO_2] and N on biomass and grain yield. We also made substantial modifications to the original DNDC (DeNitrification–DeComposition) model by introducing the electron donor–acceptor relationship under submerged soil conditions. The revised DNDC model, combined with the plant process model simulated well the redox state of the soil and the time-courses of methane flux observed in the FACE experiments. Testing of the model under different climatic conditions will improve its robustness.

5. Evaluation of Trace Heavy Metal Loadings in Arable Soils

Public concern about food safety is increasing. Some hazardous heavy metals such as cadmium (Cd) tend to accumulate in human bodies through food intake, and it is a matter of urgency to manage the risks posed by these substances. This project, supported by the Ministry of Environment for Research on Pollution Prevention and Control began in FY 2002 and ended in FY 2004. The objectives of the project were 1) to evaluate the environmental loads imposed by some heavy metals through rainfall and through agricultural practices such as fertilization and irrigation in agro-ecosystems; and 2) to clarify the dynamic aspects of heavy metal loading, such as crop uptake, solubilization, and precipitation, in soil–plant systems. The main results obtained are summarized as follows.

1) Estimation of crop uptake of some heavy metals and their loading of arable lands in association with fertilization: We developed a new technique that uses a stable isotope, ^{113}Cd, to estimate Cd uptake by crops. This enabled us to determine the residual effects of Cd contained in phosphorus fertilizers. Quantitative evaluation of Cd loading of arable lands indicated that there is no need to be concerned about the risk of Cd accumulation in the soil under common agricultural practices.
2) Heavy metal loading and crop uptake of animal manure and various agricultural chemicals used on forage crop fields: Quantitative evaluation of the flows of heavy metals indicated that the amounts of heavy metals taken up by crops are very small compared with the amounts incorporated in the form of animal manure, and that heavy metals such as zinc and copper tend to accumulate in soils.
3) Evaluation of heavy metal loading of arable soils by irrigation and rain: the Cd loading of arable soils from irrigation water was much smaller than that from rain and atmospheric deposition.
4) Influence of soil genesis on distribution and movement of trace heavy metals: We constructed a database of the distribution of trace heavy metals on arable lands in Japan. In some hilly soils, the concentration of heavy metals in the soil was closely related to the ion exchange capacity of organic matter and clay.
5) Chemical speciation of soluble heavy metals in arable soils: As the soil was air-dried, the amount of exchangeable Cd increased. Almost all chemical species of Cd in soil solution were found in the cationic fraction when soil was collected under aerobic conditions, but the anionic fraction of Cd in soil solution significantly increased when soil was collected under anaerobic conditions.

6. Studies on the risk assessment of agricultural ecosystems in the face of climate change and variability

Because global environmental change is likely to have enormous impacts on the Asian agricultural ecosystem, evaluation of the vulnerability of the system to such impacts is essential, not only for future food security but also for the sustainable development of societies in the Asian region. From these viewpoints, this study aims: 1) to use both precipitation changes projected by GCM (general circulation model)-derived downscaling methods and changes in soil moisture conditions in the crop root zone (as estimated from a water budget model) to evaluate the impacts of climate change and variation on the environment and production of major crops in China, so as to specify the most vulnerable regions and suggest mitigation strategies; 2) to improve our understanding of the distribution and variability of meltwater as a resource and its contribution to the formation of spring river discharges in Heilongjiang Province in China; 3) to evaluate the effects of global warming on soil N mineralization in the major types of Japanese paddy soils; and 4) to predict the effects of climate change on rice production in Asia using a rice growth model, and thus to suggest technologies for adaptation to future change.

We found that soil moisture conditions are tending to become drier in northeastern China, as well as on the North China Plain (Fig. 1). Agricultural water demand in southern China is projected to decrease generally, and the cropland soil–moisture deficit will decrease as a result of climate change. However, in northern China agricultural water demand is expected to increase, and the soil–moisture deficit will increase generally. Specifically, what we should be concerned about is the fact that growers of rain-fed crops on the North China Plain and

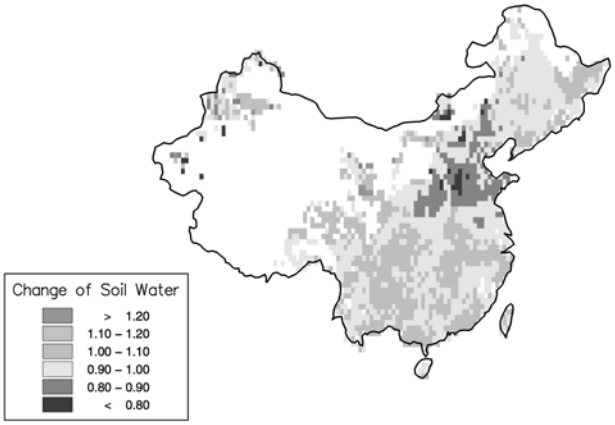

Fig. 1 Changes in normalized soil moisture (ratio of annual mean soil water content to soil water capacity in crop root zone) between 2 periods: 1946–1975 and 1976–1995.

in northeastern China will face water-related challenges in coming decades because of the expected increases in water demand and soil-moisture deficit and an expected decrease in precipitation. The effective adaptation options should include adjustment of the disproportion among plantations, forestry, and stockbreeding; large improvements in the efficiency of water use in agriculture; and an increase in the supply of water to northern China.

Next, we conducted growth and yield simulations for different rice genotypes grown under 700 μmol/mol CO_2 and different degrees of temperature rise at several sites across Asia. Figure 2 shows the results of simulations using the cultivars 'IR72' (indica) and 'Nipponbare' (japonica) at Iwate and Kyoto in Japan, Nanjing and Yunnan in China, and Ubon in Thailand as percentage yield change from the base conditions (700 μmol/mol CO_2 and 2001 and 2002 weather conditions) at each site. The model predicted, for example, higher simulated yield responses to elevated CO_2 in 'IR72' than in 'Nipponbare' at all locations under the current temperature conditions; this result agreed well with observations. Because the model assumed the same leaf photosynthetic response to CO_2 for both genotypes, the higher yield response of 'IR72' to CO_2 was caused by this cultivar's larger sink formation ability and, hence, the greater source limitation under ambient CO_2 than with 'Nipponbare'. A review of previous studies and the model simulation indicated that the anticipated global climate change associated with increased greenhouse gas production will have large and different effects on rice production in Asia, depending on the location and genotype. The model predicts that, although doubling CO_2 with a temperature rise

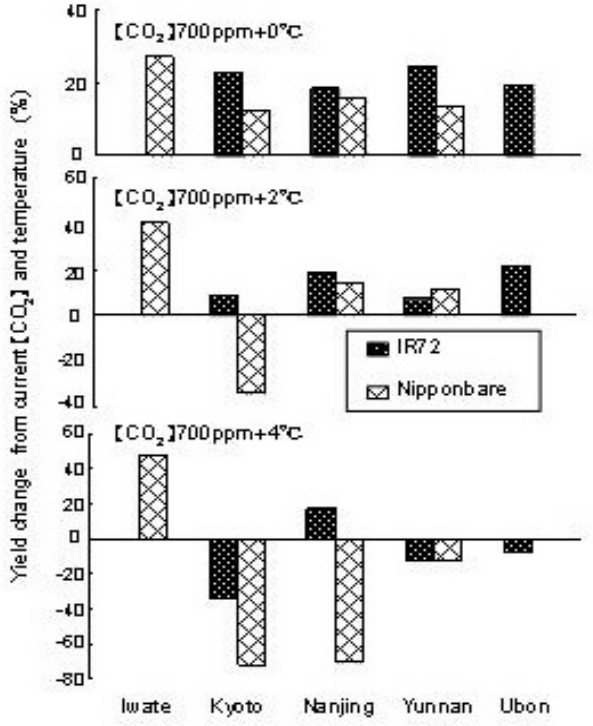

Fig. 2 Percentage yield change from the current conditions of 700 μmol/mol [CO_2] with 0, 2, and 4 °C temperature rises, as simulated for the cultivars IR72 and Nipponbare at Iwate and Kyoto in Japan, Nanjing and Taoyuan in Yunnan, China, and Ubon Rachathani in Thailand. Climate data for 2001 and 2002 at each location were used as base conditions.

of more than 2 °C will significantly increase rice yield in cool temperate areas, it will drastically reduce yield in warm temperate areas and yields of dry season rice in the tropics. The identification of adaptive technologies for mitigating the negative effects of global warming on rice production in each region in Asia is an important subject for further study.

This research project was supported partly from FY 2002 through FY 2004 by the Global Environmental Research Fund of the Ministry of Environment.

7. Use of material cycles in catchments to assess ecosystem acidification and eutrophication caused by acidic deposition

Recently in eastern Asia there has been concern about the effects of increased levels of reactive nitrogen on terrestrial ecosystems. An increase in the atmospheric deposition of nitrate and ammonium has the potential to change material flows and cause acidification and nitrogen saturation of forested catchments. We conducted a 3-year research project from 2002 to 2004 to clarify the

nationwide status of the nitrogen cycle and mechanisms of nitrogen saturation. The project used field surveys and a mass balance model and was run in cooperation with Shinshu University, Hokkaido University, Nagoya University, Tokyo University of Agriculture and Technology, The University of Tokyo, and the Forestry and Forest Products Research Institute. The research was supported by the Global Environment Research Fund of the Ministry of the Environment.

Field surveys of the nitrogen cycle were carried out in several catchment areas (e.g. Mt. Norikura in Nagano, Mt. Shirahata in Hokkaido, Tama in Tokyo). The Norikura catchment area was oligotrophic, and atmospheric input, stream-water loss of nitrogen and N_2O emission rates were low. At Mt. Shirahata, stream-water loss of nitrogen and N_2O emission rate were larger than at Mt. Norikura, although nitrogen deposition rates were almost the same. We found that the higher nitrogen output at Mt. Shirahata was caused by the severe phosphorus limitation in the volcanic ash soils. At Tama, nitrogen input and output were both much larger than in the other catchment areas. Primary production, dry weight of litterfall, and nitrogen content of leaves and litterfall were also larger. The chronic excess nitrogen input stimulated the internal nitrogen cycle and caused a larger external output.

We conducted a nationwide survey of the stream-water quality at 1278 survey points in summer 2003 to determine the current nitrogen status of Japanese catchment areas. The arithmetic mean nitrate concentration was 26.2 μmol/L, and the median was 18.1 μmol/L. Low concentrations were observed mainly in the Hokkaido and Tohoku areas. High concentrations were often observed in the vicinities of Tokyo, Osaka, the Inland Sea, and Kitakyushu. To investigate the effects of nitrogen deposition on stream-water chemistry, we used agricultural statistical data and land-use distributions to estimate the spatial distribution of ammonium emissions from fertilizers and livestock wastes. We then calculated the distribution of nitrogen deposition with a spatial resolution of 1 km by 1 km from the ammonium emission data and from existing data on the emission of nitrogen dioxide. By using a nitrogen balance model that took plant growth and nitrogen removal due to organic matter accumulation and denitrification into account, we estimated the nitrogen concentration in the stream water at each grid cell on the basis of the nitrogen deposition distribution. The estimated concentration roughly corresponded to the measured concentration ($r = 0.65$, $P < 0.001$). These findings suggest that long-term nitrogen deposition is an important factor in determining nitrogen concentrations in stream waters in natural ecosystems.