

2.8 Activities of Project Researchers

(1) Dr. Hisashi Tomemori

Summary of research

Fundamental research for sustainable agricultural development in arid land was conducted.

Recycling system of phosphate in soil-water by porous glass materials (PGM)

Phosphate which is increased by human activities is a key factor of eutrophication. In the meanwhile, phosphate is an essential element for normal growth of plants, and is applied to farmland as fertilizer to maintain high crop productivity. It is predicted that phosphate rock will be exhausted in 21st century. Therefore, the other phosphate resources are required.

We previously reported the ability of PGM to adsorb phosphate and that calcium contributed to this process (Man and Environ., 31, 11-16, 2005). And this year we examined ways of controlling the release of the heavy metal from PMG, and acquired the result expected about release control of chromium.

Research and development of hydroponics systems

In arid land, good-quality water for agriculture is difficult to get, and poor-quality water causes the salt accumulation problem by irrigation in many cases. Then, I studied hydroponic systems which prevents problems such as salt accumulation, and which can be adapted for the arid area. However, the good-quality water which can be used for hydroponics is difficult to get in arid land. So I contrived a new plastic greenhouse which had water harvest function. And tomato was actually grown using it.

Terrace was made from removing soil of a sloping ground (1.6m in height), and the single-flow type plastic greenhouse (3.5m in width and 4.5m in length) which used the cutting plane of the terrace as a wall surface was built. About 30% of rain which fell on this special plastic greenhouse was collected. And collected rain was stored in a tank installed beside the plastic greenhouse. The water stored in the tank was used as raw water for hydroponics, and provided all the water required for cultivation of tomato. Although temperature in the plastic greenhouse exceeded 40°C, the tomato grew favorably and the fruit ripened. Since it was the experiment in Japan, there were much amounts of precipitation compared with arid land, but it was confirmed that the system I designed works as a hypothesis.

Publications:

- (1) Nakazawa, R., **Tomemori, H.**, Hirano, A., Mochizuki, H., An, P. and Inanaga, S. (Aug. 2006): Effects of application of porous glass materials treated with phosphate on the growth of tomato plants and phyto-available phosphate in soil, *Soil Science and Plant Nutrition*, 52(4), 540-544.

(2) Dr. Naru Takayama

Summary of research

Geographic Classification and Monitoring of Loess Plateau by Natural Environment Factors.

-Climate, Erosion and Vegetation-

The Loess Plateau in China is semi-arid area located in E100° to 115° and N34° to 40°. This region is the front area of desertification in China. The current desertification of China, as represented by the Loess Plateau, is result of the form of agriculture at each era, the scale of economic activity, and the natural recoverability determined by precipitation and the ecosystem. This study is aiming to clarify spatially the features of climate, vegetation and erosion in the Loess Plateau by using climatological approach, satellite

remote sensing techniques and geographic information system (GIS). As a result, we showed the spatial distribution of potential vegetation of Shaanxi province. Also, we are now studying about the interaction of these natural environment elements.

Growth of vegetation is dominated from temperature and aridity level. We represented climatic factors as Warming Index (*WI*) and Aridity Index (*AI*), respectively. *WI* is the effective accumulated temperature. And *AI* is defined as P/ET_0 (Precipitation/potential evapotranspiration), so it indicates arid condition as smaller value. We used daily temperature, rainfall, sunshine duration, vapor pressure and wind speed data observed at 53 stations from 1971 to 2000, and calculated ET_0 by Penman-Monteith method (FAO, 1998). And then, monthly normals of *WI*, *P* and ET_0 was estimated by using techniques of topographic factors analysis based on Digital Elevation Model (GTOPO30), respectively. In recent, we can obtain digital vegetation maps and high spatial resolution satellite image. So, we used China land-use vector data (Liu, J and Buheasier, 2000) to know the spatial distribution of vegetation. This map is based on Landsat TM data which performed land-cover classification technique. We investigated the range of *WI* and *AI* in which the land-cover is natural forest and natural grass-land respectively in Shaanxi province by using GIS.

The distribution of *AI* and *WI* on the areas of natural forest and natural grass-land was investigated, respectively. Shaanxi province is located in typical loess plateau area. The precipitation decreases from south to north, so living of natural forest is limited near Yanan (N36.6°, E109.5°). There is significant difference in the distribution of *AI* between natural forest and natural grass-land, though there is few difference in the distribution of *WI*. In conclusion, transition of vegetation from forest to grass is caused by aridity level in Shaanxi area. In the transition belt of vegetation from the forest to the meadow (steppe), *AI* ranges from 0.34 to 0.58, approximately. The spatial distribution of potential forest area and meadow area presumed by using *AI* and *WI* was supposed. We could know the relationship between climate and vegetation in the Shaanxi area located in central of the Loess Plateau. Following to this result, we are now studying the interaction of climate, vegetation and erosion. Because, we think that our study helps the conservation of the soil and water loss by the erosion in this area.

Publications:

- (1) **Takayama, N.**, Kimura, R., Kamichika, M., Matsuoka, M. and Xing-chang. Z., 2007: Feature of Seasonal Rainfall and Estimation of Normals of Monthly Precipitation on the Loess Plateau of China. Journal of Applied Meteorology and Climatology, (Submitted).

International work shops and meetings

- (1) **Takayama, N.**, Kimura, R., Kobashi, A. and Zhang, Z., 2006: Geographic Classification and Monitoring of Loess Plateau by Natural Environment Factors. –Climate, Erosion and Vegetation-. Abstracts of CAS-JSPS Core University Program Japan-China Joint Open Seminar on Combating Desertification and Development in Inland China of Year 2006 held in (Tottori City).

(3) Dr. Hidetoshi Mochizuki

Summary of research

Water vapor movement in soils

The main objective of this study is to measure the water vapor movement in soils. We measured evaporation rate of soils by using vapor permeable membrane under several relative humidity conditions to realize the characteristics of the membrane. Yanling soil, Tottori dune sand, and Masa soil were used for the experiments. The evaporation rate of samples covered with the membrane was 10% lower than that of

not covered samples. As a result, it was clarified that the membrane prevents the vapor transport of 10%.

Development of a new method for predicting the oil content of oil polluted soil by its thermal conductivity & dielectric constant

Soil pollution by oil such as fossil fuel is the serious problem all over the world. To remediate the polluted soil, the oil content in soil must be realized. But no method which is easy for operators, and can measure it rapidly and accurately in situ, has been developed. By the measurement of thermal conductivity and dielectric constant, the oil content can be calculated by solving the simultaneous equations for the physical properties and oil content. For the Masa soil, the new method can predict the oil content quite well under the fixed temperature.

Prediction of soil erosion and productivity by EPIC model in Chinese loess plateau

The Chinese political project 'Grain for Green' will terminate in a few years. After the termination, the re-cultivation in the steep slope land by farmers will be a severe problem for the land conservation in Chinese loess plateau. To prevent from the re-cultivation, scientists have to develop and propose sustainable agricultural approaches to farmers. The soil erosion and productivity were calculated by EPIC model and implemented a field survey in the Chinese loess plateau to obtain some information on soil, water, agriculture and economics for the simulation. As a result, the agriculture on the terrace field can prevent soil erosion of 30% without decrease in farmers' income.

Publications:

- (1) **Mochizuki H.**, Koiwasaki M., Suko T. 2007. Thermal conductivity of washed Tottori dune sand with rape seed oil and modeling. *J. Jpn. Soc. Soil Phys.* Vol. 105, p.59-65
- (2) Nakazawa R., Tomemori H., Hirano A., **Mochizuki H.**, An P., Inanaga, S. 2006. Effects of application of porous glass materials treated with phosphate on the growth of tomato plants and phyto-available phosphate in soil. *Soil Science and Plant Nutrition* Vol.52(4), p.540-544
- (3) Inoue M., **Mochizuki H.** 2007. A diagnostic technique on soils, *in Dryland Science in the 21st Century: sustainability of Nature and Society*, Ed. Tsunekawa A., Kokon Shoin, Tokyo, p.84-91
- (4) **Mochizuki H.** 2007. Traditional knowledge on water management and irrigation technique, *in Dryland Science in the 21st Century: sustainability of Nature and Society*, Ed. Tsunekawa A., Kokon Shoin, Tokyo, p.84-91
- (5) **Mochizuki H.**, Koiwasaki M., 2006. Characteristic test of water vapor permeable membrane by evaporation experiment, *Annual Meeting of JSIDRE, Utsunomiya*, p.1000-1001
- (6) Koiwasaki M., **Mochizuki H.**, Suko T. 2006. Prediction Method for Oil content in Contaminated Soil, *Annual Meeting of JSIDRE, Utsunomiya*, p.978-979
- (7) Irie G., Fujiyama H., Nakajima H., Yamamoto T., **Mochizuki H.**, Miyazaki K. 2006. Soil conditioner with fiber flock, *Annual Meeting of JSIDRE, Utsunomiya*, p.942-943

(4) Dr. Ryoji Nakazawa

Summary of research

1. Recycling of phosphate in soil-plant/water system by using porous glass materials

Eutrophication in closed water system (such as lakes and ponds) has been progressed by human activities year after year. Phosphate is a key factor of eutrophication, and is released from human activities. On the other hand, phosphate is an essential element for normal growth of plants, and is applied to farmland as fertilizer to maintain high crop productivity. The shortage of phosphate deposits within 21st

century is predicted. We have been investigating the agricultural use of 'porous glass material'. As a result, we discovered the decrease in phosphate concentration in solution by coexistence of the porous glass material. Porous glass materials are recycling materials made from milled waste glass (including bottles etc.) and heat-degradable and forming reagents (such as CaCO_3). Therefore, I had already analyzed the mechanism of the decrease in phosphate concentration by coexistence of porous glass material. Porous glass material was made from waste bottle glasses and several kinds of forming reagents. The phosphate-adsorption was caused by calcium added as forming reagents. Furthermore, it is shown that porous glass material which has higher phosphate-adsorbing capacity can be manufactured by the addition of large amount of CaCO_3 . From the assay of Truog-phosphate in soil and bioassay with tomato plants, it is suggested that the phosphate-treated porous glass material contained phyto-available phosphate compared with chemical fertilizers. In addition, phosphate adsorption by porous glass materials can be controlled by their size. From the above results, it is strongly suggested that phosphate in water systems can be removed by using porous glass material, followed by reuse of the phosphate-containing glass material as fertilizer.

In this year, I revealed the following findings; 1) Porous glass materials release alkaline, and decrease plant growth. However, simultaneous application of peat with porous glass materials (phosphate-retained) improved plant growth. 2) As a mechanism of phosphate adsorption, I proposed that the diffusion of phosphate into particle of porous glass materials restricts the phosphate-adsorption. 3) The release of Cr (VI) is decreased by the use of BaCl_2 in the preparation of porous glass materials.

Publications:

- (1) **Nakazawa, R.**, Tomemori, H., Hirano, A., Mochizuki, H., An, P., Inanaga, S. Effects of application of porous glass materials treated with phosphate on the growth of tomato plants and phyto-available phosphate in soil. *Soil Science and Plant Nutrition* Vol. 52 No. 4, pp. 540-544 (2006)
- (2) Gama, P. B. S., Inanaga, S., Tanaka, K., **Nakazawa, R.** Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *Afr. J. Biol.* Vol 6 No. 2, pp. 79-88 (2007)

(5) Dr. Takehiko Ito

Summary of research

1. Conservation ecology of wild ungulates in Mongolia

Conservation and management of Mongolian gazelles (*Procapra gutturosa*) and Asiatic wild ass (*Equus hemionus*) are urgently required. Mongolian gazelles migrates hundreds or thousands of kilometers seasonally. Asiatic wild ass is more critically endangered species than Mongolian gazelles. However, details of their ecology are still unknown, because continuous tracking is difficult. The distribution area of Asiatic wild ass overlaps with that of Mongolian gazelle in south eastern Mongolia. In that area, the influences of an international railroad between Russia and China and international borders between Mongolia and China on the long-distant migratory species are concerned. To conserve animals that migrate long distances, it is necessary to know their basic ecology and influences of human activities. We, therefore, undertook the satellite tracking of the species, and are analyzing relationships between their movements and environmental factors, using remote sensing, ground survey, and geographic information systems (GIS).

We described gazelles' movements for 3 years successfully and showed a barrier effect of an international railroad on the gazelle migrations. Normalized difference vegetation index (NDVI) values

derived from satellite imagery were higher in the summer range of gazelles than in the winter ranges during summer and this relationship shifted in the migration seasons in some areas. This shift explained a reason of gazelle migration well. We also revealed one-sided impacts of the railroad on gazelles by counting gazelle carcasses on both side of the railroad. The difference would be caused by specific movement direction in autumn migration. We also showed the interannual changes of seasonal home range of the tracked gazelles, and changes of the seasonal ranges were corresponded to interannual changes of environmental condition.

We started to analyze habitat use of wild asses to compare that of Mongolian gazelles, and to present effective conservation strategies.

2. Conservation ecology of saiga antelope in Central Asia

To conserve saiga antelope (*Saiga tatarica*) inhabiting Central Asia, the first international conference of scientific research and conservation of saiga antelope was held, and I attended it and discussed current situation and future research and conservation plan.

Publications:

- (1) Ito, T. Y., Miura, N., Lhagvasuren, B., Enkhbileg, B., Takatsuki, S., Tsunekawa, A. and Jiang, Z., 2006: Satellite tracking of Mongolian gazelle (*Procapra gutturosa*) and habitat shift in their seasonal ranges. *Journal of Zoology* 269: 291-298
- (2) Ito, T. Y., Miura, N., Lhagvasuren, B., Enkhbileg, B., Takatsuki, S., Tsunekawa, A. and Jiang, Z., 2006: Migration and conservation of the Mongolian gazelle. Abstracts of the 20th Annual Meeting of Society for Conservation Biology. p17
- (3) Ito, T. Y., 2006: A report on the 20th Annual Meeting of the Society for Conservation Biology (2006). *Honyurui Kagaku (Mammalian Science)* 46: 215-216 (in Japanese)
- (4) Ito, T., 2007: Appropriate technologies in pastoralism and wildlife management. In: Tsunekawa A. ed. *Dryland Science in the 21st Century: Sustainability of Nature and Society*. Kokon Shoin, Tokyo. p. 169-175 (in Japanese)

(6) Dr. Taiichiro Hattori

Summary of research

1. Effects of silicon application on drought tolerance of crops

Application of silicon is known to enhance crop tolerance against various environmental stresses. In terms of drought tolerance, however, only few studies are available, and relationships between silicon and crop physiological processes relating to drought tolerance remain unclear. We have revealed that silicon application could enhance drought tolerance of sorghum by promoting root growth and alleviating hydraulic conductance on water pathway through plant. Thereafter, we investigated the effects of silicon application on stomatal conductance in sorghum by solution culture experiments. Sorghum seedlings raised with and without silicon application were exposed to gradually increasing light intensity, combination of sudden increase and decrease in light intensity, and water stress induced by mixing sorbitol with the culture solution. There was increased stomatal conductance with increasing light intensity and no significant difference was observed between the two silicon treatments. Under water stress, the stomatal conductance of silicon-supplied seedlings was higher than that of silicon-deficient seedlings. The higher stomatal conductance in silicon-applied seedlings under water stress was attributed to increased leaf water potential due to silicon-induced enhancement of hydraulic conductance. The results indicated that silicon application

could affect stomatal conductance in sorghum seedlings through the modification of plant water relations.

On the other hand, we also investigated effects of silicon application on growth of several temperate forage grasses (rye, tall fescue, smooth brome grass, and timothy) under well-watered and low moisture stress conditions. In conclusion, silicon application enhanced growth of timothy under stressed condition, smooth brome grass under well-watered condition, and rye under both soil water regimes. Silicon application had no effect on growth of tall fescue irrespective of soil water condition. These results indicated that there were species differences in response to silicon application even in same gramineous species.

2. Re-estimation of conventional knowledge and appropriate technologies in relation to agriculture and forestry in arid land

Current situation of agriculture and forestry in arid land were surveyed and problems related to water resource scarcity, low soil fertility, injury by disease and pest, and unpredictable drought disaster were pointed out. Then, conventional knowledge and appropriate technologies to overcome those problems were reviewed, and their strengths and limitations were discussed. In conclusion, consistent combination of conventional knowledge and appropriate technologies, such as LISA (low input sustainable agriculture) and IPM (integrated pest management), as well as careful introduction of advanced technologies including remote sensing, will be efficient.

Publications:

• Original paper

- (1) **Hattori, T.**, K. Sonobe, S. Inanaga, P. An, W. Tsuji, H. Araki, A. E. Eneji and S. Morita. : Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. *Environmental and Experimental Botany*. 60:177-182. 2007.
- (2) **Hattori, T.**, K. Sonobe, S. Inanaga, P. An and S. Morita : Effects of silicon on photosynthesis of young cucumber seedlings under osmotic stress. *Journal of Plant Nutrition*. (Submitted).
- (3) **Hattori, T.**, K. Sonobe, H. Araki, S. Inanaga, P. An and S. Morita : Silicon application improves water uptake by sorghum through the alleviation of stress-induced increase in hydraulic resistance. *Journal of Plant Nutrition*. (Submitted).

• Book

- (1) W. Tsuji and **Hattori, T.**, 2-1 Trend in dryland researches. In *Dryland Science in the 21st Century: Sustainability of Nature and Society*, Edited by A. Tsunekawa, pp. 61-66, Kkokon Shoin, Publishers Co., Ltd., Tokyo, 2007.
- (2) **Hattori, T.** and N. Yamanaka. 4-1-3 Appropriate technology in agriculture and forestry. In *Dryland Science in the 21st Century: Sustainability of Nature and Society*, Edited by A. Tsunekawa, pp. 148-157, Kkokon Shoin, Publishers Co., Ltd., Tokyo, 2007.

• Oral presentation

Hattori, T., K. Sonobe, S. Inanaga and P. An : Effects of silicon application on water relation of sorghum under osmotic stress. Workshop on silicon in plants. Kurashiki, Japan. 2006.

• Poster presentation

Hattori T., S. Inanaga, and P. An : Improvement of drought tolerance in sorghum by silicon-induced enhancement of water uptake. International Symposium of the Institute Network. Kyoto, Japan. May 2006.

(7) Dr. Wataru Tsuji

Summary of research

1. Effects of defoliation on the growth and yield in grain sorghum under soil drying conditions

In arid and semi-arid regions, it is predicted that water resources such as ground water will decrease. On the other hand, food deficit will drastically expand due to explosion of population in these areas. To solve these issues, it has been required to establish the way to conserve water resources and to increase crop productivity. So far, no resolution has still been proposed. Recently we conceived new cultivation technology which was consistent with water resource conservation and enhancement of productivity in drying soil. This is 'defoliation', which means cutting lower leaves. We hypothesized that defoliation reduced total amount of transpiration and water absorption under drought, remaining water in the soil transported to upper leaves, and improvement of water status in upper leaves enhanced total photosynthesis, finally achieved higher biomass and grain production by fewer leaves. In this study, the effects of defoliation on the growth and yield in grain sorghum under soil drying conditions were investigated using two sorghum cultivars, Gadambalia (drought tolerant) and Tabat (drought susceptible). In both cultivars, defoliated plants (cut all leaves excepting for upper three leaves) showed significantly higher photosynthetic rate and leaf water potential than control (non-defoliated) plants in flowering and maturing stages under drying soil. Water content in soil cultivated defoliated plants was also higher than that in control plants. Grain yield showed tendency to increase by defoliation in both cultivars, but not significant. These results suggested that defoliation had possibility to improve the water status in upper leaves, and to reduce water consumption in the soil regardless of cultivars. Further research to reveal the most adequate degree and timing of defoliation is needed to obtain the highest biomass and yield under drying soil.

2. Effects of seed hardening treatment on seedling emergence and growth in wheat under soil drying, and its genetic differences among varieties

Seedling establishment following germination and emergence is one of the growth processes most susceptible to environmental stresses, including soil desiccation. Unstable germination and seedling establishment due to low precipitation are one of reasons for low crop yield in arid and semi-arid regions. It was reported that subjection of seeds to wetting and redrying before sowing, which is called 'seed-hardening' treatment, enhance the emergence rate in several crops under low soil-moisture. However, genetic differences in emergence for seed-hardening effect are unknown in wheat. In this study, the effect of seed-hardening treatment on the emergence and seedling establishment in 40 wheat genotypes provided by ICARDA (International Center for Agricultural Research in the Dry Areas), and its genetic differences among wheat genotypes were examined. Only under severe drought condition, not in wetted condition, seed-hardening treatment significantly enhanced emergence rate in the most of varieties. The magnitude of seed-hardening effects in emergence was different among varieties. The variety had the higher effects showed greater seedling establishment following emergence compared with the variety had the lower one because of higher photosynthetic rate. Some genes expressed by seed-hardening treatment might be related with the enhancement of emergence and seedling establishment under drying soil.

Publications:

- (1) Hattori, T., Sonobe, K., Inanaga, S., An, P., **Tsuji, W.**, Araki, H., Eneji, A.E., Morita, S. Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. *Environmental and Experimental Botany* 60: 177-182, 2007.

- (2) **Tsuji, W.**, Araki, H, Inanaga, S., An, P. Is water uptake efficiency of deep roots of two sorghum cultivars differing in drought tolerance different under topsoil drying? *Plant Production Science* (submitted).
- (3) Eneji, A. E., Inanaga, S, Muranaka, S., Li, J., An, P., Hattori, T., **Tsuji, W.** Comparison of silicon sources for dry matter yield and nutrient uptake among four species of the gramineae under deficit and adequate irrigation. *Journal of Agronomy and Crop Science* (submitted).
- (4) **Tsuji, W.** and Hattori, T. 2007. Research wind in dryland science. *Dryland Science in the 21st century: Sustainable of Nature and Society* (ISBN978-4-7722-3105-3), ed. Tsunekawa A., Kokon Shoin Publishers Co., Ltd., Tokyo, 58-66 (In Japanese).
- (5) Yamada, S and **Tsuji, W.** 2007. The system to extend appropriate technology. *Dryland Science in the 21st century: Sustainable of Nature and Society* (ISBN978-4-7722-3105-3), ed. Tsunekawa A., Kokon Shoin Publishers Co., Ltd., Tokyo, 175-182 (In Japanese).

(8) Dr. Xiangjun Li

Summary of research

1. Nitrogen accumulation in soybean following defoliation

The effects of defoliation on soybean [*Glycine max* (L.) Merr.] growth and yield have been well studied, but relatively little is known about the nitrogen accumulation after defoliation. A field experiment was conducted to examine how soybean recovery their nitrogen accumulation following defoliation. The indeterminate cultivar (Tousan 69) was planted in greenhouse and two defoliation treatments (nondefoliation and 67% defoliation) were applied when soybean were at R₂ (plants had at least one flower in the two uppermost nodes) stage. At 0, 15, 30 and 45days postdefoliation (DPD), plants were destructively sampled to measure dry weight, nitrogen accumulation and nitrogen fixation. The seed yield and its nitrogen concentration were also measured at maturity. The results showed that seed yield and its nitrogen concentration were not affected by defoliation. Although defoliation reduced temporarily soybean dry weight and amount of nitrogen accumulation at 0 and 15 DPD, defoliated plants was able to completely recovery both of dry weight and amount of nitrogen accumulation at 30 DPD. There was less difference in nitrogen concentration between defoliated and nondefoliated plants, indicating that defoliated plants recovered their nitrogen accumulation as dry mass recovered. Recovery of nitrogen accumulation in defoliated plant was due to complete recovery of N₂-fixing ability, and also related to improvement on nitrogen absorption after defoliation.

2. Effect of cytokinin application on growth recovery after defoliation in soybean

It is well known that soybean can compensate for defoliation through two mechanisms: compensatory leaf regrowth and delayed leaf senescence. And also, the difference in defoliation-tolerance among various soybean cultivars was resulted from the difference in leaf regrowth ability and leaf senescence after defoliation. As one type of plant hormones, cytokinins have physiological function to promote leaf expansion and to delay leaf senescence; it may be assumed that cytokinin application could promote the growth recovery following defoliation in soybean. To test this hypothesis, a pot experiment was conducted in environmentally controlled growth chamber. The determinate cultivar (Enrei) was exposed to two defoliation treatments (nondefoliation and 67% defoliation) and four cytokinin treatments (0, 0.001mM, 0.01mM and 0.1mM, spraying 6-Benzylaminopurine solution for four times) when soybean were at R₂ (plants had at least one flower in the two uppermost nodes) stage. The interactive effects of defoliation and

cytokinin application on leaf expansion, photosynthetic gas exchange and dry mass production were determined. The dry weight and leaf area was significantly reduced by defoliation at 36 days after defoliation. 0.01mM and 0.001mM cytokinin application did not affect both of dry weight and leaf area at this time; while 0.1mM cytokinin application significantly reduced dry weight and leaf area. Less increase in dry weight of defoliated plants with cytokinin application indicated that cytokinin application did not promote the recovery following defoliation. However, photosynthetic data showed a tendency to promote compensation for defoliation. The defoliated plants with cytokinin application had higher photosynthetic ability than defoliated plants without cytokinin application. It is not clear why the higher photosynthetic ability in defoliated plants with cytokinin application did not promote dry mass production in this study; therefore, future study is essential.

3. Development of indicator system for the extent of desertification in arid region of China

This study is one of Core University Program. The field investigations were conducted in inland (Mu Us sandy land) and coastal region (salt accumulation soil, Cangzhou city, Hebei province) of China. Based on field data, the relationships between plant community and soil environment were revealed by clustering and ordination analysis, and new indicator system for desertification was proposed finally.

Publications:

- (1) **Xiangjun Li**, Ping An, Shinibu Inanaga, A. Egrinya Eneji and Kenji Tanabe. Salinity and defoliation effects on soybean growth. *Journal of Plant Nutrition*. 2006.29:1499-1508.
- (2) Ping An, Shinobu Inanaga, Nanwen Zhu, **Xiangjun Li**, Hassan M. Fadul and Messaoud Mars. Plant species as indicators of the extent of desertification in four sandy rangelands. *Afr. J. Ecol.*,45:94-102.
- (3) Yuanrun Zheng, Yi Yu, Glyn Rimmington, Lei Zhang, Ping An, Guangsheng Zhou, **Xiangjun Li**, and Hideyuki Shimizu. Response of 4 dominant species in central Inner Mongolia to air temperature and soil moisture. *Journal of plant research*. (submitted).

(9) Dr. Atsushi Suetsugu

Summary of Research

1. Spatial distribution of *exoQ* and *mcpK* genes in sandy soil with preferential flows

Spatial distribution of soil microorganisms has been considered to form the distribution of vegetation, because soil microorganisms include symbiont or harmful species for the plants. The regional growth of soil microorganisms are promoted by many factors including nutrients, water, gases, temperature, light, host species, and signal compounds. The distributions of some of these factors can be estimated by using climatic or topographic data, but preferential flows include chaotic process to generate complex moisture conditions in soils. Furthermore, many species of soil microorganisms can actively migrate or lower the cell motility, while abiotic substances (e.g., clay particles, dead cells, solutes, or immiscible liquids) are passively transported by the preferential flows. The positive chemotaxis to water (hydrotaxis) has been found as an important strategy of cyanobacterial species to survive in desert soils (Garcia-Pichel and Pringault, 2001). Other genetic systems common in drought tolerant species (e.g., compatible solute production, exopolysaccharides production, spore formation) lower the cell motility. Therefore, the distribution of soil microorganisms in the natural growth media should be explained by both of hydrological principles and microbial regulation systems. In the present study, metagenomic detection of exopolysaccharides production (*exoQ*) and chemotaxis (*mcpK*) genes of nitrogen-fixing microorganisms was conducted at sandy soil with two types of preferential flows (fingering flow and bypass flow) and

homogeneous flow. Microbial incubation experiment with the three types of flow was conducted with originally developed aseptic Hele-Shaw systems. Universal PCR (polymerase chain reaction) primers for *exoQ* and *mcpK* were newly developed with highly similar sequences among *Rhizobium sp.*, *Bradyrhizobium sp.*, and *Agrobacterium tumefaciens*. For efficient design of universal probes, a software program that finds the sequences with the highest or the lowest melting temperatures within a universal probe was developed. The melting temperatures of deoxyinosine-containing universal primers were estimated with the nearest-neighbor thermodynamics of deoxyinosine pairs in DNA duplexes (Watkins and SantaLucia, 2005). These primers with GC-clamp worked well even though the conserved domains are as short as 190 base pairs. Metagenomic DNA samples from compost-amended sandy soil were collected at twelve locations for each type of flow. Denaturing gradient gel electrophoresis (DGGE) patterns and nucleotide sequences of the major bands will be shown in the next year.

2. Functional gene array analysis of salinity responses of *Bradyrhizobium japonicum* USDA110 and microbial community in compost samples

Drought tolerance of microorganisms is a key factor to introduce plants, and is provided by many types of genetic systems. Some of those systems are considered not to work simultaneously, because somnucell (dormant cell) formation inhibits chemotactic cell movement. The complex blending of drought tolerance gene expressions in environmental microbial community should be clarified with a practical method, because most of the whole-genome sequences of soil microorganisms are still unknown. Functional gene array is composed of limited number of probes with annotated functions, and is used for the response analysis of a specific organism or microbial community sample to various kinds of environmental stresses (e.g., Dennis et al., 2003). In the present study, the salinity responses of *Bradyrhizobium japonicum* USDA110, a drought sensitive symbiont strain with soybean, and microbial community in compost samples were investigated with a functional gene array. The functional gene array is designed for 39 genes including both of *Bradyrhizobium* specific and universal probes for *cheA*, *cheB*, *cheW*, *mcpK*, *motA*, *pomA*, *flhA*, *fliI*, *fliM*, *flaA*, *flgE*, *flgH*, *flbT* (chemotaxis and cell motility), *exoZ*, *expG*, *otsA*, *betB*, *putA*, *ectC*, *dapA* (cell structure and osmotic regulation), *ntrB*, *ntrC*, *fixJ*, *fixK*, *fixL*, *fixR*, *nifH*, *nifQ*, *nifA*, *nodA*, *nodD1*, *nodD2*, *nodZ*, *nosR*, *nosZ*, *nirK*, *norB*, *napA* (nitrogen metabolism), and *rDNAs* (for microbial community structure characterization). The method of total RNA recovery from metagenomic microbial community in compost samples was optimized with macro-scale (10g) bead-beating homogenization in guanidine-Denhardt buffer and subsequent purification by Trizol-chloroform and illustra RNAspin mini kit (GE Healthcare, Co., Ltd., Tokyo, Japan) with on-column DNase I digestion. The microarray spotting and hybridization were conducted by Ecogenomics Ltd. (Kurume, Japan). The results will be presented in the next year.

Publications:

- (1) Suetsugu, A. 2006: Spatial distribution of nitrogen-fixing/denitrifying microorganisms in soils with preferential flow, *18th World Congress of Soil Science*, pp. 366-367.

(10) Dr. Toshihiko Kinugasa

Summary of Research

In 2006, I started a long-term research programme about the effect of increased nitrogen deposition on vegetation and nomadism in Mongolian grassland as described below. This research is supported by Grants-in-Aid for Scientific Research from Japan Ministry of Education, Culture, Sports, Science and

Technology and will continue until 2009.

Effects of increased nitrogen deposition on the vegetation of Mongolian grassland and its potential for grazing

The amount of nitrogen fall-down from the atmosphere to the biosphere (nitrogen deposition) is increasing globally due to increased fossil fuel consumption and chemical fertilizer application. Galloway et al. (2004) reported that the amount of annual nitrogen deposition in Asia was around 1000 mg/m² in early 1990's. They estimated that the area receiving annual nitrogen deposition over 1000 mg/m² has increased and the annual nitrogen deposition can reach as high as 5000 mg/m² in some regions by 2050. As nitrogen is one of the macronutrients essential for plant growth, increased nitrogen deposition affects plant primary productivity (Vitousek et al. 1997). However, increased nitrogen deposition can also decrease plant diversity (Stevens et al. 2004).

Mongolian grassland is a steppe spread out in the north of central Asia and is utilized for grazing by nomads. As grazing potential would depend on the productivity of grassland, it is sensitive to any global change that affects grasslands. Galloway et al. (2004) have estimated that the amount of annual nitrogen deposition in this region in 2050 is likely to increase by about 1000mg/m² over that in early 1990's. Even in arid region, where the primary factor limiting plant growth is water availability, about 1000mg/m² increase in nitrogen deposition has been found to enhance plant growth (Alon & Steinberger 1999). Thus the increase in nitrogen deposition may influence grazing potential in Mongolian grassland through change in grassland productivity and composition. Increased nitrogen deposition may also affect vegetation recovery in Mongolian grassland damaged by severe grazing pressure.

In the present research, nitrogen is artificially applied to Mongolian grassland to assess the effect of increased nitrogen deposition on grassland vegetation and productivity and ultimately the grazing potential. The effect of increased nitrogen deposition on the recovery of grazed grassland is also evaluated. The study therefore aims:

- (1) To analyze the effect of increased nitrogen deposition on the growth of the vegetation and productivity of Mongolian grassland.
- (2) To evaluate the change in livestock carrying capacity of Mongolian grassland as a result of an increase in nitrogen deposition.
- (3) To evaluate the effect of increased nitrogen deposition on the recovery process of grazed grassland.

Materials & Methods

Study site: The study site is in Bayan-Unjuul, which is located in the center of Mongolian grassland. This study area is typical dry steppe, about 150km south west of the capital Ulaanbaatar. Annual mean temperature and annual mean precipitation are 0.3°C and 165mm, respectively.

Plot design: Three experimental plots of 19m x 15m each were established in the grassland. Each plot has two main treatments, grazing and non-grazing. The non-grazing plots will be surrounded by fence to prevent animals. In each main treatment, three nutrient sub-treatments to vary nitrogen supply are established: application of no nutrient solution, application of nutrient solution and application of water. Thus, there are six treatment combinations in the experiment.

Nitrogen fertilization: Annual amount of nitrogen fertilization is 1000mg/m². A nutrient solution containing required amount of ammonium nitrate (NH₄NO₃) would be sprayed by a sprayer in June, July and August when mean monthly precipitation is relatively high (25-40mm). To evaluate the effect of water applied with nutrient solution, the treatment with only water is also included.

Measurement: Plants would be harvested three times in the growing period (from June to August). At each harvest, six subplots (0.5x0.5m) would be randomly chosen from each experimental treatment. After recording observed species and counting the number of each species, above ground parts of plants would be harvested and leaf area of each species would be measured. Dry mass and nitrogen content of samples would also be measured. Using the obtained data, the followings would be analyzed:

(1) Long-term effect of nitrogen deposition Increased plant growth due to increased nitrogen deposition would affect the composition of the vegetation in the successive years. Assessing the change in grassland production and species composition, long-term effects of nitrogen deposition on the vegetation of Mongolian grassland would be analyzed.

(2) Effects of nitrogen deposition on grazing potential As the livestock grazing depends on the palatability of the grassland vegetation, the change in grassland productivity may not always reflect the actual livestock carrying capacity of the grassland. Therefore, to analyze the effect of increased nitrogen deposition on grazing potential, palatability of the vegetation to the livestock in Mongolian grassland would be surveyed in the literature. Combining data on vegetation and palatability, the effect of increased nitrogen deposition on grazing potential would be estimated.

Publications:

- (1) Kinugasa, T., Shinoda M. and Tsunekawa A. (Mar. 2007): How does increased nitrogen deposition affect vegetation and grazing potential of Mongolian grassland? The 54th Annual Meeting of the Ecological Society of Japan

(11) Dr. Takayuki Kawai

Summary of research

1. Groundwater recharge system at sand dune

When resolving the groundwater recharge system in sandy area, it is necessary to regard the soil moisture movement. Therefore, we have been observing the vertical movement of soil moisture for a long term at natural slope of Tottori sand dune, Japan. As a result, it has been understood that the vertical soil moisture is not distributed evenly, and it is classified into wet layers and dry layers.

On the other hand, the sand dune has the fine lamination structure even an aeolian dune or a hydro one. This lamination structure is a fine-veined pattern that caused by each sand particle arrays methodically, and intricate lamination is the one of the geological features of sand dune. Then, we compare the vertical soil moisture patterns with the lamination structure. As a result, it is clarified that the soil moisture is also discontinuous in the point where the lamination structure is discontinuous. This indicates that the groundwater recharge system is influenced by the lamination structure.

2. Groundwater-Environment analysis with groundwater flow sound

Because of existing hydrological survey needs huge time and budget, a detailed groundwater exploration in arid land is difficult. Then, the development of the new observation method that is suitable for the investigation in the arid land is demanded. We paid attention to the groundwater flow sound, which is a generic name of various sound waves generated when groundwater moved.

When the groundwater flow sound is measured in the Tottori sand dune with a developed sensor, a high correlation is seen in sound pressure and the groundwater level. It is suggested to be able to estimate the groundwater level from the groundwater flow sound under a homogeneous geological condition. Moreover, the groundwater flow sound is measured also in a Mongolian plain. The local points where sound pressure is large or small are unevenly distributed also in the plain with little topographic features change. It is suggested to be able to presume groundwater flowing quantity that originates in the geologic structure.

Publications:

- (1) Tada, Y., Fujita, M., Tsutsumi, D., Okumura, T., Honda, N., Yanagisawa, J., **Kawai, T.**, Koyama, K., 2006: Estimated accuracy of water path distribution at mountain slope using the groundwater flow sound. Proceeding of Japan Society of Erosion Control Engineering, pp. 148-149 (in Japanese)
- (2) **Kawai, T.**, Kamichika, M., Tada, Y., 2006: Relationship between lamination and soil water content at Tottori sand dune. Proceeding of Japan Society of Sand Dune Research, pp. 2-3 (in Japanese)
- (3) Koyama, K., Tada, Y., Fujita, M., **Kawai, T.**, 2006: The dangerous points estimation of slope collapse using the groundwater flow sound. Proceeding of Forest road Research, pp. 26 (in Japanese)
- (4) Koyama, K., Tada, Y., Fujita, M., **Kawai, T.**, 2006: The simple diagnosis method at slope with groundwater flow sound. Survey. Proceeding of Mountain Conservancy Research, pp. 115-120 (in Japanese)
- (5) **Kawai, T.**, Tada, Y., Shinoda, M., 2006: The groundwater-environment measurement in arid land using the groundwater flow sound. Agricultural Meteorology of Chugoku and Shikoku, 19, pp. 2-3 (in Japanese)