Reviewers' comments and our responses

The result of evaluations of three reviewers and responses of program leader to each reviewer's comments is as follows:

1) Action class judgment of the research program

[B]

2) Comments and suggestions for the research program

Generally, the research topics selected are important and will certainly contribute to better management of soil fertility and plant mineral nutrition. The research program has quality research adhering to good research approaches and procedures. Opportunities exist in some cases for research to transition from a 'trial and error' approach to a more systematic collection of data for use in the development of predictive abilities. However, there are several issues that should be considered, that would strengthen the science and overall excellence of the programs.

Excellent progress has been made in developing analytical methodologies such as for soil phosphorus and available soil nitrogen. A number of 'products' from the research are now at the stage of meriting deployment to end users. [a)] Thus a plan should be developed for deployment of research 'products' to end users through partnerships, such as with local research and extension organizations. More evaluation of the research, particularly the applied research, needs to be done with farmers. [b)] Research to increase plant availability of nutrients from sources other than fertilizer should include the quantification of appropriate reductions in fertilizer use to achieve balanced crop nutrition and protection of the environment. [c)] There is a lack of research to enhance mechanistic understanding of plant-soil interactions and provide conclusive information. In future studies, attention should be given to clarification of the mechanism of action of different aspects investigated. For example, NARO can initiate an extensive soil and plant survey study, at least for the vegetables. This type of survey study would generate highly useful data that can be important for designing of the future research topics under "Integrated Soil Management and Sustainable Production" and also for better understanding of the nutritional quality of the vegetables harvested.

Due to huge problems with high usage of P fertilizers, current research programs should also involve investigations about the role of seed-enrichment with P in reducing usage of P fertilizers. Majority of raw materials for the P fertilizers is imported from overseas. [d)] Analyzing those materials for Cd and other metals before their use in development of P fertilizers is important.

We suggest that more basic research is needed to complement and strengthen the excellent

applied research. The merit and significance of the research could be enhanced through greater exposure of the research to the international research community. [e] In case of publications of the results, priority should be also given to international peer-reviewed journals. Some of the science that is being conducted could be published in high impact journals. The links and integration among activities within a project could be further clarified and enhanced. There needs to be more collaboration among scientists in NARO, and particularly with scientists in other organizations and institutes. We strongly suggest that such collaborations should be intensified.

(Response to the underlined comments)

a) We have a plan for the dissemination of research 'products'. It is necessary to test the applicability of the soil tests in various regions in order to make fertilizer recommendations, so we have collaborated with the extension centers of Ibaraki, Nagano, and Aichi prefectures to encourage the use of the simple evaluation method for available nitrogen. We are also testing the available phosphorus method in the farmers' fields in Chiba prefecture. We will make further efforts to collaborate with more local research/extension organizations in order to encourage wider use of the soil tests we have developed.

b) We showed the results of omitting basal phosphorus application in cucumber culture based on the soil test, and of a 30% reduction of phosphorus application in soybean culture by using the biological function of the indigenous arbuscular mycorrhizal (AM) fungi. We will make further efforts to achieve appropriate reductions in fertilizer use through effective use of nutrients in soil, livestock manure, and green manure and through utilization of biological functions.

c) We recognize the importance of studying the mechanism of soil-plant interactions. We will strive to elucidate the influence of soil properties on the effect of using pelletized manure, and the mechanism of ethylene evolution reduction in organically grown apples.

d) In Japan, we have a regulation related to heavy metal concentrations in fertilizers, which means that fertilizer companies check the heavy metal concentrations in the phosphate rocks they use. The requirement for heavy metal analysis in this research program is therefore relatively low.

e) Even though we achieved good results from our research, in certain cases we submitted papers to Japanese journals only, rather than to international journals. We will promote basic research and make further efforts to actively contribute articles to international journals.

3) Comments and suggestions for the 6 research projects

(1) Soil management based on efficient use of nutrients in soil by using new methods (151a1)

The goals of this project are excellent and developing a user friendly and rapid method for assessing available P and N will be of great value to farmers. The development of a simple and quick test may contribute to better soil management in terms of efficient use of mineral nutrients. The opportunity now exists for greater deployment of developed soil tests to farmers and local extension services. The development of a detailed plan for deployment is encouraged. However, before introduction of such quick tests, [a)] it is essential to examine suitability of this method in diverse soils differing greatly in plant available P concentrations. The soil tests need to be calibrated by running trials at several locations with different soils and inclusion of plant analyses, i.e., a linkage of plant N and P to soil levels would be important. Also, research is merited to determine the relationships of farmers' practices and the history of fields with soil test results and to calibrate soil tests with fertilizer requirements for upland crops and rice. [b)] Opportunities exist for research to transition from a 'trial and error' approach to systematic collection of data for use in the development of predictive abilities. For example, research on localized P application with plug nursery medium has resulted in valuable findings. Research with systematic collection of data is now merited to determine optimal P sources and amendments for a given soil type and season.

The studies on the role that K additions have on the uptake of radiocesium (Cs) from contaminated soils are interesting and could prove quite important. [c)] However, more basic studies are needed to understand the reaction mechanisms through a special attention by foliar spray of K fertilizers. In case of the experiments dealing with minimizing Cs contamination of plants, a special attention should be given to foliar spray of K fertilizers such as KNO_3. In addition, in calculation of the transfer factor for rice and other plants, besides seed-Cs, also shoot-Cs content should be considered.

(Response to the underlined comments)

a) We think a rapid soil test helps farmers to consider their own fields' status and leads to P fertilizer usage. Accumulation of soil available P is a common problem, especially in greenhouse cultivation. For this reason, we first developed the rapid soil P test for soils with high rates of available P accumulation and the soil test was calibrated for greenhouse cucumber at three prefectures through a joint research project. Because of its sensitivity, this test might be inconvenient for on-site analysis on soils containing less available P. Ongoing study is aimed at improving the test at different sites with soils with lower available P rates.

b) We recognize that a collaborative relationship with local government agricultural research stations is necessary for systematic data collection. We will promote joint research with the research stations through the acquisition of cooperative funds.

c) We recognize the importance of basic studies on the reaction mechanism of Cs uptake by crops. We will pass on these important comments to the members of the research program 510, which was established in 2012 and has been conducting research and development in relation to the aftermath of the nuclear power station accident.

(2) Rhizosphere technology for using nutrient dynamics in crop rotation in cold regions (151a2)

The effect of crop rotation on P requirements for crops is noteworthy. The results obtained indicated positive effects of previous cropping with mycorhizal plants on yield and mycorrhiza colonization of the tested plants after AM host plants. However [a)] the interpretation of results and development of recommendations for management of AM fungi should consider the effects of soil P on AM fungi and the large variability in the benefits associated with AM fungi. The practical value of AM fungi in reducing the need for P fertilization needs to be tested over the long term. [b)] The research should additionally consider the financial analysis and technical feasibility of crop rotations using AM fungi host crops to reduce P fertilizer requirements in current and possible future crop rotations.

[c)] Since mycorhizae has diverse of beneficial effects besides improving root P and Zn uptake, it was important to show both leaf P concentration and shoot P content (total uptake values) of the experimental plants. [d)] The research should consider that increases in the availability of P from soil, such as from phytic acid, must be accompanied by reduced inputs of P from fertilizer and/or organic amendments for protection of the environment. It would be important to extend the phytic acid part of the project by using a germplasm of *Lotus japonicas* to study genotypic variation in root phytase activity under P deficient conditions. [e)] In regions with low soil temperature like in Hokkaido, starting short term projects investigating role of seed-enrichment with P in seedling development and seed yield would be important.

(Response to the underlined comments)

a) The management method for soybean cultivation that we recommended was designed considering the relationship between the effects of AM fungi and soil P availability. Dispersion of the effects was also considered to limit the reduction ratio of P fertilizer to 30%. Although we already have rough data for AM colonization rates when using AM fungi for this purpose,

we should be able to clarify our results following further research.

b) Soybean has been cropped after AM fungi host crops in some current crop rotation systems. In these cases we don't need additional financial analysis because there is no additional cost, as only the function of indigenous AM fungi is utilized. We will consider economical and practical aspects when we develop a new crop rotation system, especially when we use AM fungi host green manure crops after non-host crops.

c) Although we didn't have enough time to describe our results for shoot P content in the International Review Meeting, we have measured shoot P content for all test samples and confirmed that the main factor in the effectiveness of AM fungi is promotion of P uptake. We will try to study the diverse beneficial effects of AM fungi in next mid-term.

d) In future we will show that increases in soil P availability by new management methods, such as the phytic acid utilization method, must be accompanied by reduced inputs of P from fertilizer and/or organic amendments.

e) We will start discussions about conducting research on the techniques of seed-enrichment with phosphorus in our next mid-term plan.

(3) Upland soil management in warm, high rainfall regions (151a3)

The research on determination of soil pH(KCl) and P fractionation of composts reflects quality in scientific approach and methods. However, information on the amount of P added in the experimental soils through application of manures is important for understanding and interpretation of the results presented. a) A plan for use of the methods in agriculture in Japan is required to increase the merit and significance of the research. This plan should, for example, indicate how a method to better delineate nonallophanic and allophanic Andisols would be used for better targeting of appropriate management for soils. b) The novelty of pH measurements in KCl should be explained because the soil pH(KCl) has been used for many years to provide a measure of exchangeable Al.

The research on pelletization represents a noteworthy scientific contribution. Experiments are needed to explain the mechanism(s) of the positive effects of pelletized compost on better plant growth and better root P uptake. c) The mechanism(s) for increased P uptake needs to be better elucidated. Systematic research is now merited to quantify the effects of soil properties, such as P-fixing capacity, on the benefits of pellets relative to other sources of P.

(Response to the underlined comments)

a) Strong soil acidity has frequently injured pasture growth in the area studied; therefore, elucidation of distribution and properties of nonallophanic Andosols in this area was required. Based on our achievements, we plan to improve pasture production in this area as follows: i) by transferring information about the distribution and simple discrimination of nonallophanic soils to farmers, agriculture instructors and official staffs by holding seminars and publishing publicity leaflets, ii) by developing simple estimation method of lime requirements for the neutralization of nonallophanic Andosols, and iii) through development of an acidity improvement technique using regional resources such as ash from chicken waste.

b) A major objective of this study was to solve a problem with soil management in Japan. Exchangeable acidity y1[#], which has been used as the index of exchangeable aluminum in Japan, is problematic because it is laborious and therefore difficult to use in practice. The pH(KCl) soil test is a method of evaluating soil acidity using extraction conditions similar to those of y1; however, precise estimation formula between y1 and pH(KCl), based on analysis of various and numerous soils, had not been available. We discovered this formula for the first time by analyzing representative upland soils in the Kyushu Okinawa Region. We also demonstrated that soil acidity management using pH(KCl), which can be easily measured by farmers and agriculture instructors, is possible. Now we are planning the development of a soil acidity management method for the control of common scab in potato fields using pH(KCl) in cooperation with regional agricultural research institutes.

c) We observed that phosphorus bioavailability differed between bulk compost and pelletized compost. In accordance with the valuable suggestions provided, we will investigate the fate of phosphate in composts by an incubation study using various soils and sources of composts.

#: Exchangeable acidity y1[1)] is the amount of 0.1 mol/L NaOH solution to neutralize 125 mL of 1 mol/L KCl soil extract (250 mL solution to 100 g air-dried soil). In Japan, critical values of y1 for acidity improvement are defined in soil testing; e.g., Niigata Prefecture recommends to maintain y1 in grassland soils at 3 or lower. In addition, y1 value is used as criteria in soil classification; e.g., $y1 \geqq 5$ is one of the criteria of Nonallophanic Andosols[2)].

1) Saigusa, M., Shoji, S. and Takahashi, T. 1980. Plant root growth in acid Andosols from northeastern Japan: 2. Exchange acidity Y1 as a realistic measure of aluminum toxicity potential. Soil Science, 130, 242-250.

2) Classification Committee of Cultivated Soils. 1996. Classification of cultivated soils, third approximation, p.26, National Institute of Agro-Environmental Sciences, Tsukuba, Japan.

(4) Nutrient dynamics model and agricultural technologies for water quality conservation of watersheds (151b0)

The automated pulsating drip irrigation (APDI) reflects an output of research with a practical application. a) The APDI systems seems to be effective in reducing N_2O flux, N fertilization amount and application of water. However, a detailed data for the cost-benefit analysis is needed.

The findings of reduction of greenhouse gases with different systems is encouraging. The modeling that is being conducted is first-rate and most useful. The research appears to contain considerable findings some of which might benefit from even greater examination and interpretation. As an example, the reported higher emission of greenhouse gas (nitrous oxide) from compost than chemical fertilizer in the analysis of farming technologies represents a finding with implications that are not much considered in the results presented for the project. New technologies, that show promise, are being tested and validated, and there are some collaborations that are ensuing with other research organizations. b) The integration among activities within the project should be further clarified and enhanced for success and significance of the research. Increased integration of activities within the project might increase the probably of success and significance of the research.

(Response to the underlined comments)

a) The initial investment expense of the APDI system is one fifth the cost of a typical drip fertigation system, or 200,000 yen/10a. The average sales income per farmer who introduced APDI was 2,321,000 yen/10a/y, which was 23% higher compared with the sales income of 1,886,000 yen/10a/y of farmers using conventional irrigation. Therefore, the introduction expense of APDI is recoverable in one year.

b) Our collaborative relationship with other research organizations is ongoing, and an integration evaluation including LCA and economy is being carried out. In developing the environmental load reduction technique, we plan to improve the accuracy of estimates for such things as fertilizer nitrogen leaching rates. Based on the results, our technique for evaluating water quality change following the introduction of load reduction technology will be improved. And then, in the development of the integrated evaluation technique, development of the technique in terms of economic value will be enhanced.

(5) Assessment and management of soil biological fertility (151c0)

The development of new biological indices for crop production is a noble goal of the project. The investigators are collaborating with other institutes on the research. a) In order to increase the

significance of the research, information should be provided on how the biological indices will be used and how they relate to production of crops or the productivity of agricultural systems. [b)] Considerations should be given to how a more sensitive method for ciliates with DNA extraction and how a DGGE analysis for fungal populations will be used to develop management recommendations. It would be important to use the methods developed here in the 151a2-project dealing with the effects of previous cropping with mycorhizal (AM) plants on growth and yield of the plants. One question that needs attention is to combine the biological indices with chemical profiles.

Research on the effects of solar sterilization on ammonia oxidizing bacteria reflects good scientific methods. [c)] In order to increase the relevance of the research, biological solutions like thermophile nitrifying bacteria could be compared with other management options such as addition of nitrate.

(Response to the underlined comments)

a) We will take crop productivity into consideration when we develop the biological indices. The biological indices will be one of the new key factors in comprehensive soil diagnosis, which, for example, will give new organic farmers valuable information on soil management relating to changes in nutrient dynamics among conventional fields, transit fields and stable organic fields.

b) Our protocols should be applied. They are:

To assess soil bio-fertility during continuous application of organic materials and to propose soil suitable management for new organic farmers.

To assess the effect of solar sterilization on microbial populations and their nutrient dynamics.

And to establish an assessment and management manual on pathogenic risk for stable asparagus production in upland fields.

c) Application of manures after solar sterilization is partly expanded in some regions. We plan to compare the handling and cost performance of the conventional manure spotting method and our revised protocol.

(6) Plant nutrition for efficient management of nutrients using metabolomic analysis and endophyte utilization (151d0)

The array of studies being pursued and the technical quality of them is excellent. However, [a)] there seems to be little connectivity between the studies, and it was not clear how they are going

to address and/or answer research questions in other programs. The title of the project 151d0 is, therefore, too general and not focused.

Metabolomic analysis of apples reflects use of good scientific methods. [b)] Research could further examine the factors related to less sweetness and flavor in organic apples. The results obtained with organic apple should be further studied to understand why in organically grown plants ethylene evolution is impaired. In the experiments with isolated endophytes, molybdenum (Mo) nutritional status of plants would also be particularly important. [c)] The research on nitrogen-fixing endophytes could additionally consider effects of soil nitrogen supply capacity, soil fertility, and N fertilizer on the amounts of nitrogen fixed. The mechanism of organic N release is important to pursue, but further studies are needed. More consideration should be given to how the research results will be used for improved management of soils and better use of composts. [d)] The symptoms described for boron (B) deficiency of broad bean also occur very similarly under copper (Cu) deficiency. Therefore, it is suggested to provide also Cu concentrations of those seeds with less and high blackening rate.

(Response to the underlined comments)

a) We have promoted connections between the studies in our project when necessary, although the connectivity among the studies is not necessarily strong enough. In fact, we have applied for a patent on a new type of endophyte isolated through joint research by members on metabolomic analysis and endophyte utilization. In the design of the array of studies in the next mid-term plan, we would like to pay more attention to cooperation among the studies.

b) We have continued our analysis of apple flavor and its link with cultivation technique. In particular, inhibition of ethylene emission under organic cultivation is a subject of importance. We will focus on evolution of ethylene and the flavor-related gene as well as flavor profile to discover the full scope of the system of external signals - gene expression - in relation to the metabolic response of apples.

c) We will make further efforts to examine the effects of the status of soil nutrients, with special attention paid to N fertilizer, on the infection and nitrogen fixation of endophyte.

d) The research groups from Kagoshima University and Kagoshima Prefectural Institute for Agricultural Development have already reported that boron concentrations of the seeds of blackening broad bean are lower and the concentrations of the other elements, including copper, are not different when compared with normal seeds, and that the blackening disorder is concluded to be due to boron deficiency resulting from experimental water culture without

boron and boron foliar application.