

CONSERVATION AND SUSTAINABLE

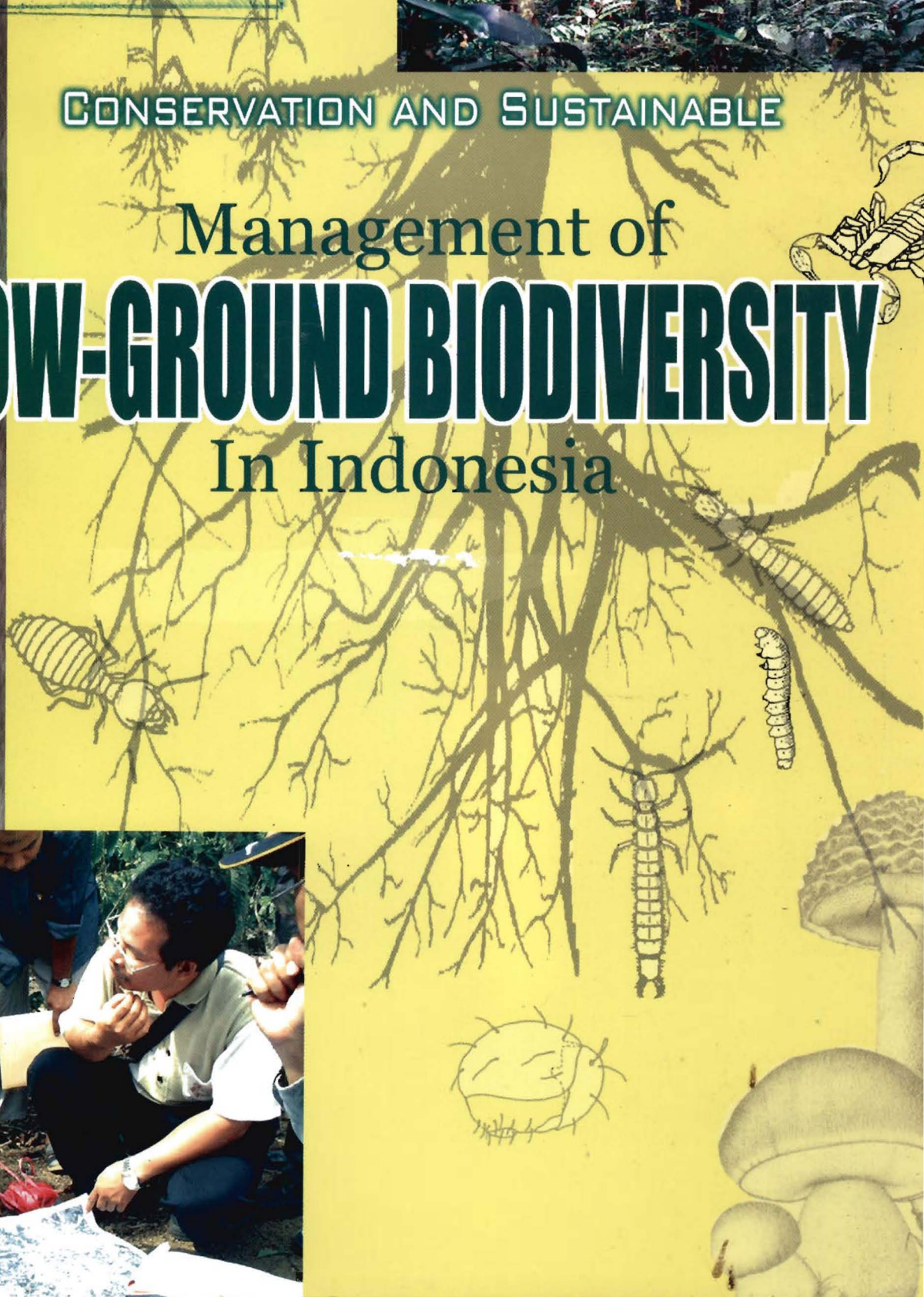
Management of

# BELOW-GROUND BIODIVERSITY

In Indonesia



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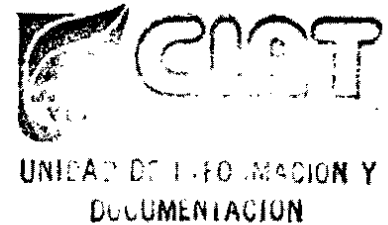
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CONSERVATION AND SUSTAINABLE MANAGEMENT OF  
**BELOW-GROUND BIODIVERSITY**  
**IN INDONESIA**

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## PREFACE

Below-ground organisms consist of microbes (bacteria, fungi, protozoa) and macrofauna (invertebrates). Their diversity is very high, especially in the tropical regions. They provide services which are important for the existence and functioning of natural ecosystems, for instance in 1) driving the nutrient cycling; 2) regulating the dynamics of soil organic matter, soil carbon sequestration and greenhouse gas emissions; 3) modifying soil physical structure and water regimes; 4) enhancing the amount and efficiency of nutrient acquisition by vegetation (mycorrhiza and nitrogen fixing bacteria); and 5) contributing to plant health through the interaction of plant pests and pathogens with their natural enemies. These services are essential for the sustainability of all terrestrial ecosystems including agro-ecosystems. However, data on below-ground biodiversity (BGBD) are limited. This could be as a consequence of ignorance, lack of research (methods), deforestation and/or agricultural intensification.

While there is need to conserve BGBD, there is also need to evaluate whether such conservation brings costs or benefits in terms of agricultural productivity and added value to any ecosystem service. Answering these questions requires research carried out at the farm level to the landscape level. We argue that conservation of BGBD provides win-win solutions for productivity and environmental protection. Outputs achieved via manipulation of above-ground components (result to increase in productivity, biological control affectivity, etc) and those manipulated by below-ground components (results to improvement in soil structure and water regimes through the activity of soil fauna, efficiency of nutrient cycling through microbial regulation; reduction of greenhouse gas emissions/soil carbon sequestration due to regulation of decomposition processes; and increase effectiveness of biological control of soil-borne pests and diseases).

As part of the global effort to conserve and manage BGBD and in connection with the Convention on Biological Diversity (CBD) that has been ratified by Indonesia, a national core project team has been formed and taken the initiative to implement the "Conservation and Sustainable Management of Below-ground Biodiversity (CSM-BGBD)" programme in Indonesia. Seven such national teams (Indonesia, India, Uganda, Kenya, Ivory Coast, Mexico, Brazil) coordinated by the Tropical Soil Biology and Fertility-Centro Internacional de Agricultura Tropical (TSBF-CIAT) in Nairobi have joined to form a global project team work of CSM-BGBD. The work is done within the framework of the GEF/UNEP project. As a precursor to the implementation of the project, a startup workshop was held in Wageningen University in The Netherlands in August 2002. As a follow-up a technical workshop was held at Sumberjaya-Lampung (Indonesia) in February 2003. One of the points recommended in the Sumberjaya meeting was that each country held a national workshop to launch the programme. The Indonesian team held the workshop in 30<sup>th</sup>—31<sup>st</sup> May, 2003 at Bogor, West Java. This book is one of the outputs of the workshop.



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## **BELOWGROUND BIOLOGICAL DIVERSITY: SURFACING ITS IMPORTANCE ABOVEGROUND**

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### **Introduction**

The implementation of the Convention on Biological Diversity (CBD) has not given adequate attention to the belowground biological diversity. The Decision III of the third COP (1996) has given very limited accounts on soil organisms. The indicative list of thematic areas, included only soil micro-organisms and nematodes.

By looking back at the achievements of the CBD implementation, one may disclose the parts of the CBD provisions that have been implemented, and which others have not been realized. What is more important is what Indonesia has achieved in the implementation of the CBD. It is a necessity to know which field and sectors have been involved in the act and which factors have contributed to such status and situation of the achievements.

Disclosing the situation will help determining the plan and designing the efforts to overcome the constraints and the weaknesses in implementing relevant CBD provisions. It is important that strategy and action plan developed and implemented be based on the past experience and the expectation for the future. All of this consideration will be the starting point in developing further field activities. By such strategy, action plan, field programs and activities, the direction and the design of the programs can be determined.

After ten years of the CBD, the world has made some achievements in the implementation of the Convention, by developing thematic work programs (COP, 1996), on marine and coastal biological diversity, inland water biological diversity, forest biological diversity, and agricultural biological diversity. This discussion will be focused on the agricultural biological diversity, since this is most closely relevant to the scope of the theme in this workshop.

Conferences of the parties have been conducted as many as 6 times, and the 7th will be organized next year in Kuala Lumpur, Malaysia. SBSTTA meetings have taken place 8 times, and all of these meetings have been conducted in Montreal, Canada. Including *ad hoc* group meetings, the relevant CBD meetings have documented the conclusions and recommendations that can be used for monitoring the progress of the CBD implementation. It has been covering some issues, including identification and monitoring, access to genetic resources, intellectual property rights, biosafety, alien species, dry lands and sub-humid lands, mountain ecosystems transfer of technology, including biotechnology, and ecosystem approach.

The last World Summit on Sustainable Development (WSSD) formulated results covering almost anything concerned with human being and environment. The most important aspect related to bio-conservation is biological diversity. It is stated that the importance of biological diversity in the sustainable development as a whole and the poverty levitation becomes the basic element of human welfare and human life, and the cultural integrity on this planet. The main target of Johannesburg Conference as far as biological diversity is concerned, is to minimize biodiversity loss due to unsustainable use, which is related to poverty. This objective is planned to be achieved by the following strategy (WSSD, 2002).

- To include the objectives of the CBD in all sectoral programs and policies,
- The CBD provisions should be the guidelines, especially by applying the ecosystem approach in wider sense,
- Recognition of the rights of the local communities as the owner of traditional knowledge, innovation and practices,



- Traditional and local people should be encouraged to participate in making decision and policy related to the application of their knowledge,
- Equitable sharing of the benefit, as an integrated part of the CBD three-side objective is to be based as the guiding lines of the implementation of the CBD provisions,
- Consideration on Cartagena Biosafety Protocol and Global Taxonomy Initiative.

Comprehensively, in Indonesia, the planning and program development to achieve the objective of the CBD as described above, should include all stakeholders concerned with the use of biological diversity. From the government sector, it should concern the research and academia and sectoral departments. It is also expected from the law and legal aspects, economy, public welfare, community settlement, and information and communication. The last but not the least is the involvement of the environmental aspects. From the private sector, the industries are the other stakeholder that should be concerned. The traditional and/or local communities will be one of the important determining sectors in the success of the sustainable management of biological diversity. Public at large, directly or through the non-governmental organizations, is also important sector to be expected to concern with the management of biological diversity.

So far work and activities have been focused on aboveground biodiversity and concerns started to grow towards marine biodiversity. These biodiversity components are relatively more easily recognized and, therefore, readily usable. Their morphological characters will lead to their recognition, and in consequence, they have better accessibility. Identification is easier to do, and therefore, the analyses of detailed contents have better starts. The aboveground biological diversity utilization is more practicable. From these facts, all efforts so far is to equip human resources and other resources, such as financial and physical facilities, by capacity building (including institution building), are only directed towards the management of aboveground and recently marine biodiversity.

Contrary to the above facts, belowground biological diversity still need much more attention. In many practices and facts, especially in agriculture, the components of the belowground biological diversity have shown their important roles. The deeds of these components are shown in nutrient cycling, action and coordination in the decomposition of organic matters, energy and material transfer, bioindication, and maintenance of soil porosity. Therefore, important as it may be, belowground biological diversity still needs to be explicitly surfaced, so that its importance is disclosed.

Better planning and utilization according to human's direction have to be started now. Many sectors and stakeholders must be involved in this effort, be it governmental, private, and other non-governmental ones, such as local communities, farmer associations, as well as individual researchers, according to their respective capacity. At present, issue on organic farming is strongly launched in Indonesia. To really apply this system, the life networking in the belowground biotic components must be understood.

### Implementing The CBD

Article 7 of the CBD is on the *Identification and Monitoring* of components of biological diversity important, especially, for its conservation and sustainable use. As consequences, parties are required to identify the components of biodiversity important for its conservation and sustainable use.

There are other items that have been continuously addressed under the agenda of the COPs. For biosafety, Cartagena Protocol on Biosafety has been issued. The Jakarta Mandate is developed to guide the management of marine environment and resources. The Secretariat has also adopted Biodiversity Day to remind everyone to give concerns on biological diversity. There have been issues on access to genetic resources, and they have been addressed to result in the adoption of *Bonn Guidelines on Access to Genetic Resources and Fair and Equitable Sharing of the Benefits Arising out of their Utilization*. Alien species received its treatment to alert parties to be aware of the threats exposed by these biotic components.

The Commission on Sustainable Development in monitoring the Agenda 21 has come to organizing a summit conference as the World Summit on Sustainable Development (WSSD). This South African meeting in September 2002, resulted in the decisions of so many aspects of development, and one of them is on the conservation of biological diversity. Unsustainable agricultural practices have resulted in the large-scale degradation of agrobiodiversity and habitats through the destruction of biotic and abiotic resources. Somewhat little fortunate is that progress is being made in many regions of the world in implementing biological diversity-friendly agricultural practices, especially giving impacts on soil conservation.

Based on such consideration, an indicative list of thematic areas has been developed. In land resources, some of the areas may be related to the conservation of the belowground biological diversity, such as in soil erosion control. However, in conducting case studies, only soil microorganisms have been given further considerations. In thematic program on agricultural biological diversity, there is an indication on the awareness that soil biological diversity has role in the conservation of agrobiodiversity in particular, and biological diversity as a whole in general. The CBD conferences have addressed the importance of this biological diversity, so as to develop basis for action.

The implementation of the CBD as a whole is in slow motion. This is apparently due to the lack of understanding and awareness on the existence and the meaning of convention on biological diversity. Unfortunately, this is the case of the many supposedly to be concerned departments and sectors. The roots of this situation could be the ignorant attitude of these departments in the governmental sector. Biological diversity is not just a form of biota, but rather its contents, roles and benefits (in material, financial, social, cultural, spiritual matters, etc.) that will need the concerns of all sectors and departments. Involvements of other sectors, such as industries, local and traditional communities, as well as public at large, is of the same importance with the other sectors of stakeholders. The benefits of having concerns from all sectors will be immensely felt by the entire nation.

Many of sectors, especially outside of conservation circle, that is limited to forestry and agriculture, have never considered that conservation of biological diversity is the concern with aspects, such as economy, public welfare, law, culture, politics, information and communication, transportation, trade, and whatever constitutes human life. Many of the sectors are close to ignorant to the existence of the CBD that will determine the fate of the nation. This ignorance in many instances creates conflict of interest between sectors. The mining sector will be in the interest that is contradictory to the interest of the forestry sector. The settlement sector is not running in the same direction as the agricultural programs. The regional governments have their own aims in their commitments. Although the national government is supposed to be directing to a common goal, in reality, the situation is different. Each of the sectors considers its mission the most important.

The unhappy situation in the implementation of the Convention on Biological Diversity, for the purpose of conserving biological entities for the benefit of mankind is also augmented by the political instability. The unstable political situation in the country will have two direct impact on the conservation, namely (1) not enough time allocated for the meaningful and effective implementation program of bio-conservation, and (2) that the ever-changing government will always change its policies, and consequently will affect the management of natural resources, including biological resources and biodiversity.

### **Agrobiodiversity: Its Issues**

There have been many international conferences on biodiversity, which discuss not only its conservation but also its sustainable utilization. Moreover, the importance of biodiversity for the livelihood of traditional communities has gained more attention than before the CBD. Surprisingly enough that only few international meetings have been dedicated to agrobiodiversity, a special group of biodiversity that plays a direct role to human being.

Many are in the opinion that agrobiodiversity comprises only of crops and domestic animals. With this coverage FAO has dealt with agrobiodiversity since its inception. Therefore, discussion on agrobiodiversity is often considered appropriate to be under the FAO's wings. In the era of CBD,

however, agrobiodiversity has a broader concept than crops and domestic animals. Agrobiodiversity include all crops and livestock, as well as their wild relatives, and all interacting species, such pollinators, symbiosis, pests, parasites, etc. (Qualset *et al.*, 1995). Thus, great ranges of organisms above and belowground that play role in agriculture are included in agrobiodiversity (Wood & Lenne, 1990).

Most developing countries depend on agriculture for their economy. The same holds are true for Indonesia. Almost half of its population lives in rural areas and agriculture is their main activity. Though the average landholding is low, the government was able to introduce modern technology for rice farming in late 1960s. Not all farmers were in position to adopt the new technology in which irrigation is a must. Moreover, many types of agroecosystems do not fit for rice farming. Instead, many crops are grown together in mixed systems, which from agrobiodiversity point of view differ from place to place.

Various agroecosystems have developed according to natural conditions, culture, as well as crop appropriateness. In some places the inclusion of livestock in the systems is done. Home garden as one of the agroecosystem types is well established in Indonesia. In such a garden not only many crops are grown but also tree crops and domestic animals are taken care of. Earlier studies have shown that more than 100 species of plants are found in such a system. Farmers are aware of the importance of soil fertility for their crops. Therefore, green manure and dung are employed to enrich and maintain soil health before artificial fertilizers were introduced. They recognize pests and diseases that are harmful to their crops. They also notice organisms that are beneficial for their crops. Yet only recently the whole system is dealt scientifically.

One component of the new rice technology is the high yielding varieties (HYVs). By accepting these varieties farmers quite often abandon their own varieties. In this way the old varieties are no longer in use and in the end they are no longer available. The erosion of these traditional varieties started to attract the attention of the world community when the Green Revolution was accepted in many developing countries. FAO together with well-known foundations responded to such a concern and efforts to save genetic resources of crops began. It soon became apparent that not only rice genetic resources was in danger to being lost but all major crops such as wheat, maize, potato, and cassava experienced the same fate.

It seems obvious that many concern with crops as the main component of food production. All associated components such as fertilizers, pesticides, irrigation, and soil conditions are often considered secondary. Therefore, belowground biota and their role, important as it is, have not been recognized until recently.

Unlike the rest of belowground biota, nitrogen-fixing bacteria has been the subject of many studies. The role played by this group of bacteria in crop production has been realized since the very beginning of modern agriculture. Recently, mycorrhiza's role in tree crop plantation is recognized.

### **The Belowground Biodiversity**

Most of the belowground biological diversity has two unattractive characters. Firstly, they are not easily recognized for they are too small to detect. Secondly, they do not have spectacular and beautiful appearances. Compared to the grandeur appearances of many or most of the aboveground biological diversity, the former group is less inviting to the interest of most of the scientists. As it is shown in the lists of speakers in this workshop, the treatments of the subjects of studies is very sporadic. Mostly they are dealing with microbes, and much less on invertebrates, and it is believed that, judging from the titles of the subjects, the scope of the discussion is not purely the inventory of biodiversity as such. There is still a very vast space that needs to be filled up.

An overview on the components of belowground biodiversity has been made, and it shows that in many practices and facts, especially in agriculture, the components of the belowground biodiversity has shown their important roles in many respects. Nutrient cycling, action and coordination in the decomposition of organic matters, energy and material transfer, bioindication, and

maintenance of soil porosity, are just the deeds that have been given to the success of agriculture, especially in its productivity aspects (Adisoemarto *et al.*, 1997; Suhardjono *et al.*, 2000).

Belowground biodiversity do not live exclusively. There are interactions among these two "groups" of biodiversity. Decomposition activities of the belowground biodiversity will depend on the types of aboveground partners that will be contributed to the former group to work with. Transfer of energy and mineralization that is conducted by the belowground biodiversity will have no value unless used by the aboveground components, and the mineralization will not be in progress unless the raw materials are supplied by the aboveground components. Pests and diseases caused by the belowground components of biodiversity are directly related to the aboveground components. There are many ways of the relationships of the below- and the aboveground biological diversity.

The vast coverage of soil organisms as the components of belowground biodiversity plays innumerable roles. Albeit, the understanding of these components is very limited. Inventory in the wider sense is needed for the purpose of utilizing the existence of these components. Overcoming the problems in many aspects of agriculture, agronomy, and pest management, for example, will need studies in general but comprehensive inventory, including the distribution of the belowground biodiversity in relation to the aboveground components.

### **The Winning Team: The BGBD Project**

It is generally agreed that information on biodiversity richness at the global level though inadequate at the moment, is available (GBA, 1995). Therefore, in conference of the parties to the CBD VI (The Hague), the world community set a target for the world that by 2010 the rate of biological diversity loss will be significantly reduced. However, in order to come to an agreement on what it means by "biodiversity loss" and thus reducing its rate accordingly is not an easy task. The meeting in London recently provided a working definition as follows, "biodiversity loss is the long term of permanent qualitative or quantitative reduction in components of biodiversity and their potential to provide goods and services". With this definition then the scientific community can develop indicators to measure the loss. It was also agreed that the base line rate of biodiversity loss should be based on the decade of the 1990s rate. However, when looking at specific indicators where data is available for a longer time series, these will be considered on a case-by-case basis.

As mentioned earlier less information is available for BGBD. Therefore, the project proposed by the group of belowground biodiversity (BGBD) is so timely in accordance with the 2010 target of the CBD. The Global Environment Facility (GEF), as the mechanism for the implementation of the CBD's programs, is in position to supporting projects that enable parties of the CBD to work together to solving common problems.

The process from planning of the project, to the submission to the GEF, and finally to the approval by the GEF takes a long time. However, these scientists from seven countries who are concerned with BGBD have endured the waiting. The financial support from the GEF is indeed necessary for the group if the group should make the difference in providing evidence for the importance of BGBD in agriculture. Each participating country is basically agrarian, rich in agrobiodiversity, but lacks of resources to really do something significant.

The project on *enhancing the conservation and management of BGBD important to sustainable agricultural production in tropical landscapes undergoing land conversion and/or intensification* is an objective with a broad coverage. Linking such an objective with the 2010 target of the CBD it becomes obvious that the methodology to collecting data is set as a priority. Once the methodology is developed, agreed and tested the data on the BGBD in many sites of tropical countries will be no doubt available. Moreover, land use maps of the selected areas together with the intensity of their use will be one of the outcomes of the project.

There are number of ways to measuring aboveground biodiversity. To mention some of them are extinction based such as that done by IUCN, or area based as in the Millennium Goals, or

richness based as done by Conservation International with its Rapid Biodiversity Assessment. It is realized, however, that organisms not yet recognized by science cannot be measured. For the BGBD the project will concentrate on selected number of groups of the soil biota that have quantitatively significant functional roles. From the list of organisms presented in the proposal the project is aiming at a big picture of BGBD, not only looking at the richness of the species but also the services that they provide.

A network of scientists in seven participating countries will implement the project. With a good management these scientists who have conviction to delivering information on BGBD to other fellow scientists and policy makers will certainly provide what they promise. However, competence in biodiversity in many related fields is indeed necessary to support such a conviction. The proponent of the project is well aware of this fact. Therefore, capacity development is set as one of the outcome.

Another ingredient for making the project a success is a commitment of all the scientists in the network. In Indonesian language there is no word "commitment". Only recently the word has been absorbed to the Indonesian language the way the words president, parliament, surprise are readily used. However, Indonesian scientists who join the project are no doubt will show their commitment as their fellow scientists from other countries.

### **Sustaining The Team Spirit**

A project planning is always time consuming since the success of the project is closely linked with a good planning. However, it is also a common phenomenon that in proposing a project quite often the proponent is over optimistic with time frame for the project completion. The more the number of scientists participating in the project the more likely that the project cannot meet with the dead line of completion unless an experience project coordinator is in place. The BGBD group is well aware of this problem and thus the formation of various committees to ensure the project coordination.

Working in a team is a challenge especially if the project is a multidiscipline in nature such as this project of the BGBD. Moreover, the institutions in which scientists are working spread over seven countries. It is true that distance is no longer a limiting factor for scientists to communicate their progress, yet it is not an easy job for that who has the duty to coordinate the works. Reading the BGBD proposal carefully it seems that a dedicated group of scientists is in place. It will not be a surprise if a teamwork between them soon will flourish.

Achieving a success by a team of scientists is much easier than maintaining it. In order to be successful in cooperative or collaborative efforts the word networking is considered a must nowadays. Within a network there is equality among the members both in rights and duties. It means that each member should contribute something to the system without some one is giving him/her instruction. There are quite a number of existing networks on various activities that the BGBD group can learn why one network is more functioning than the others.

Certainly capacity to carry out activities of this project varies from country to country and from institution to institution. Although the same footing in implementing the project is expected for every member, however, it is well thought in the project proposal that capacity enhancement is aimed. At the end of the project not only the results of the group will be useful for scientific as well as for practical purposes, but each participating member will have an added value in his/her field of interest.

Compared to aboveground biodiversity, the number of scientists working with BGBD is less. Therefore, if the project is successfully implemented, there will be no doubt that the success will attract others to join. A special attention should be paid to students in the universities who are still looking for opportunity in life. BGBD in general is more microscopic than the aboveground biodiversity. Therefore, more efforts are needed to show what the BGBD is all about to the coming scientists.

The BGBD project is just starting. As early as this stage it is worth exploring how the activities are going to be sustained long after the project is ended. BGBD as a whole is under studied. Three to five years of research is not enough to unravel the mystery of belowground life. A clear vision to continue activities beyond the life of the project will help the members to work in a long-term basis. The

CBD set up the 2010 target, i.e., reduce significantly the biological diversity loss. Certainly the BGBD group is not going to ignore this target in its work program. Moreover, after Johannesburg each country tries to talk about the targets on poverty, on water sanitation, on sustainable production and consumption, on management of the natural resources base, etc. If and when the BGBD group is able to formulate the connection of its activities with those targets, then the chance of its survival is even greater because each country will take the BGBD group seriously in its agenda of development. A regional institution with a long-standing achievement in aboveground biodiversity may act as the focal point of the BGBD group.

### From Research to Policy

The role of science in development was discussed at length in the World Summit on Sustainable Development. Science based policy decisions are the ultimate goal of the works of scientific community for society if its contribution to sustainable development will be intended. To achieve such a goal a partnership between scientific community and society should be in place. However, at present the partnership is not well developed. Hence, scientific research is often considered as useful only for science for the sake of science but not for development. Therefore, ICSU (International Council of Scientific Unions) as an outstanding union of national science academies and international scientific union expressed its commitment in Johannesburg to make science more policy relevant.

One of the BGBD project objectives is recommendation of alternative land use practices, and an advisory support system, for policies that will enhance the conservation of BGBD. Such an objective is planned to be achieved by organizing meetings, workshops and consultations to report and review the results of sustainable and replicable management practices for BGBD conservation. Within the word conservation covers social and economic benefits. Thus the group of BGBD is indeed prepared to cater advice on good management of agroecosystem to all concerned parties (farmers, scientists, environment and land use planners). A wide range of target audience that is rather ambitious in nature.

To be appealing to policy makers the BGBD should respond to the sustainable development wake-up call, i.e., to WSSD goals. Among the goals are reducing hunger, reducing poverty, combating desertification, improving clean water accessibility, and restoring forests. The five objectives of the BGBD certainly fall into those goals. The question is how to package the research reports so that they can be digested by the policy makers easily.

It does not need to be emphasized that agriculture is a sector in most developing countries that has a vital role in meeting the basic needs of human population. To many small farmers agriculture is also a source to generate income. Therefore, the link between above- and belowground biodiversity as stated in the project proposal becomes essential. Since one of the target audience of the project is farmers and farmers rely on aboveground biodiversity for source of income then time frame to catering the results of the project to them is crucial. It is true that BGBD provides important services to the land on which aboveground biodiversity is managed, yet short term benefits for farmers will determine as to whether or not they will accept the best practices offered by the BGBD group.

In small agricultural systems various uncertainties operate so that harvests cannot be assured at the end of farming seasons. Bad weather, resource availability and market forces are just a few factors that influence uncertainties. The results of BGBD projects should be able to reduce such uncertainties if farmers are expected to absorb the offered options. For well established farming systems any new technology find its way to be absorbed by farmers if it coupled with the right policy and incentives. However, in newly developed agricultural land, such as that around the forests, in which farmers have no long-term experiences, new technology may have difficulty to spread.

BGBD loss keeps on going while research is still in implementation. Human activities in the agricultural areas have negative impacts as well as the positive ones. Of the negative ones are the damage to the soil structures. Application of excessive synthetic fertilizers and pesticides will alter the balance of the soil composition. This damage in turn will destroy the biotic components in the soil. Unfortunately this loss has happened before anything is done to understand the condition. Efforts to reduce this loss may be reduced though researches, in agricultural practices as well as in



understanding the existence of BGBD. In so doing, the understanding will be improved, and the treatment to the biota will be enhanced.

At present situation, the knowledge on belowground biological diversity, as looked from some aspects, shows uneven level. The diversity aspect in Indonesia is limited in groups as well as the geographical areas covered. In the progress of time, the interests are dwindling down, and the quality is following the fate of the interests. Successful agriculture, especially the ever increasing switch to organic farming, will need knowledge on belowground biological diversity, including their roles in the management of the soil environment. This need can only be fulfilled if the human resources as actors in doing research in such area reach a critical mass. Formal education, especially in the taxonomy of belowground biological diversity, is very much needed. Talking about taxonomy, it will not only cover the fixed classification as it is taught at present in this country, but rather the true taxonomy that can produce taxonomic information as needed by the end users. This information will be much needed in developing the database of the intended biological diversity, showing the role of its components, behaviors, capability, and the treatment to give accordingly. For fulfilling this need, taxonomy should be treated hand in hand with the study of genetics, physiology, biochemistry, ecology and other related disciplines for revealing biodiversity component characters and ability.

Results and findings of such research, very limited in number and coverage, are scattered in many media, including the gray ones. The publications on this subject are limited not only in the number and coverage, but also in the forms of the publications. In the future, opportunity is open widely for the studies of belowground biodiversity, in almost every aspect, especially the basic ones. This opportunity will come true if the required resources will be provided so that the critical mass will be reached. Only with this effort, which is meticulously planned and designed, the objectives may be achieved.

One worrying fact is about what will happen at the end of the project. Continuous funding may not be in hands; thus the project activities may also be no longer in place. In the project proposal there is an optimistic spirit on the sustainability of activities. The BGBD group realizes the importance of continuing its activities beyond the project's life. Therefore, a not shy promotion by the group to other potential donors should be started from now, not later. Such a promotion should be attempted not only once by at any event that the member of the group attends. It will be sad to see the great effort on BGBD stop, while a lot are yet to be done.

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## ARTHROPODS IN INDONESIA SOIL

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### Introduction

Before discussing about biodiversity, definition of "belowground" habitat has to be agreed beforehand. In this paper we define that "belowground" represents part of terrestrial ecosystem including soil surface (mulch), humus, soil and cave. "Belowground" is the most important habitat in terrestrial ecosystem which has not yet gotten much attention, especially about properties and potency of its diversity. One element of this diversity that requires attention is soil arthropods.

Whether in number of species, individual or their biomass, soil arthropods have predominated terrestrial ecosystem (Erwin, 1988; Wilson, 1988), which in number of species reaches an estimate of between 30 and 50 million. Besides, soil arthropods have been recognized to live in various soil environments and play primary roles in soil ecology processes (Collins and Thomas, 1991). Wilson (1987) proves that 93% of total animal biomass in one hectare in wet rain forest of Amazone is arthropods. Research in Canada mentioned that 75% from 1 million species of soil biota is arthropods (Biological Survey of Canada, 1991). In short, diversity of soil arthropods has provided a very high and wide potency from the biogeographical problems to their ecological roles for deeper research interests, including in Indonesian area.

Although their potency is very big, research on soil arthropods in Indonesia in general is still very low, even no one has explained in detail diversity of arthropod species in certain soil habitat. Even though information on soil arthropods in Indonesia is scarce, this paper will review results of research that have been documented and steps to do in the future.

### Ecological Roles of Soil Arthropods

Terrestrial ecosystems, especially soil, are characterized by fast and dynamic change, either naturally or through intervention. Consequently, soil inhabiting arthropods also experience these changes, both their species and population. Base line data of these diversity changes are needed, although data of this kind have not been available yet in Indonesia. Investigation on species richness is important to monitor and simultaneously explain their roles. Roles of arthropods need to be recognized to stimulate research on survey and monitoring as well as knowledge of roles of soil arthropods in Indonesia. These include their contribution in or as: (1) decomposition processes (Behan-Pellitier, 1993; Kimmins, 1987), (2) microorganism dispersal, (3) energy transfer and mineral movement (Biological Survey of Canada, 1991), (4) bioindicator, (5) soil porosity and aeration, (6) pest (Kalshoven, 1981), and (7) ecosystem functioning.

### Research on Soil Arthropods in Indonesia

In general, research on soil arthropods in Indonesia is very scarce and is generally conducted at natural forest ecosystems, and only very few at agricultural ecosystems.

#### *Based on habitat type*

Based on investigated habitat types (Figure 1), there are only a few publications at the preliminary levels, including 23 series in forest habitat, four series in plantation habitat, six series in Kart-Cave habitat, and two very common publications.

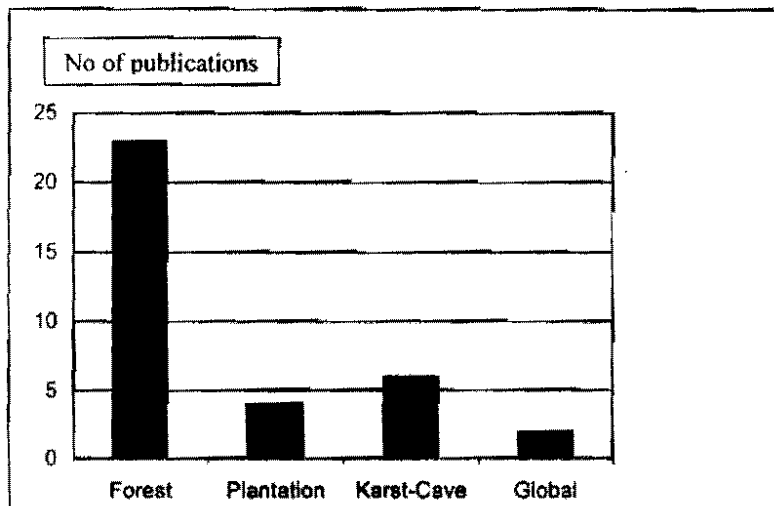


Figure 1. Research on soil arthropods in Indonesia based on habitat types.

This condition is discouraging, considering broadness of habitat diversity in Indonesia, but research on the different habitat types is very scarce. This needs more attention from all of us to encourage research in this area. This research is badly needed for optimal and sustainable land use.

#### *Based on discipline*

Based on scientific disciplines, there are 20 publications at the survey level, not species characterization of soil arthropods (only morphospecies), and need to be rechecked. Three publications explain a very common ecology and 12 publications are on taxonomy limited to Collembola (Figure 2).

Research on ecology, even at survey levels, is limited by weaknesses in taxonomy area. A number of survey studies collide with species identification of soil arthropods collected; thus, separation is only based on differences in external morphology. It can be said that no taxonomist is interested in one of the taxonomic groups of soil arthropods in Indonesia except Collembola. This has limited research on soil arthropods in Indonesia.

#### *Based on taxonomic groups*

Based on taxonomic groups investigated, there is also only a few studies conducted in Indonesia (Figure 3). Here, there are only 17 studies investigating Collembola, followed by insect (nine series), and arthropods general (eight series). Almost all of the publications neither explain existence of new species nor depict diversity at a certain habitat. Research is still limited to certain taxonomic groups in limited areas. Thus, the number of noted or identified Collembola species is about 300 (Suhardjono, 1988; Suhardjono, 1989; Suhardjono and Deharveng, 1992; Deharveng and Suhardjono, 1994; Deharveng and Suhardjono, 2000; Yoshii and Suhardjono, 1989; Yoshii and Suhardjono, 1992a-b). If the number of Collembola species is estimated to reach 6000 worldwide, in Indonesia there should be at least about 600 - 1000 species. By field experience, Indonesia is rich of species included in the families of Paronellidae and Entomobryidae. Both families have very high species diversity.

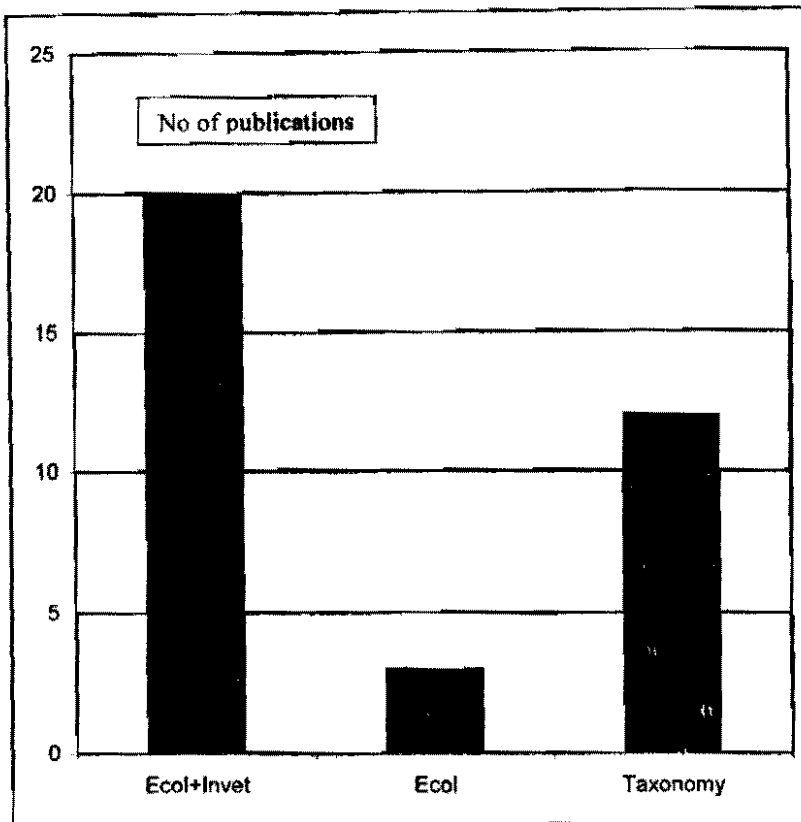


Figure 2. Research on soil arthropods in Indonesia based on scientific disciplines.

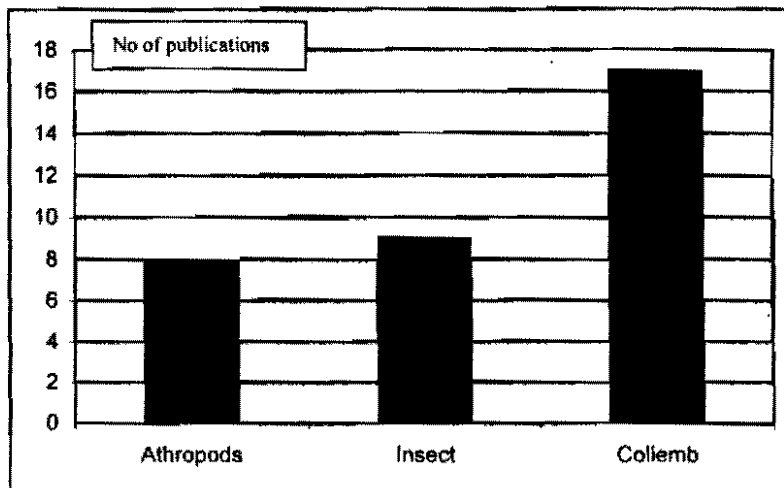


Figure 3. Research on soil arthropods in Indonesia based on taxonomic groups.

Human resources responsible for taxonomy have been one problem in some countries (Noyes, 2000 pers. com.), including Indonesia. This is a challenge to all of us. Without taxonomic expertise, it will be difficult to understand species diversity of soil arthropods. Taxonomic trainings at early levels are required to be developed, and encouragement to all-young biologists to have an interest in taxonomy area needs to be created.

#### *Based on region*

Despite widespread distribution of the Indonesian regions, research on soil arthropods is limited only to certain regions. Research conducted in Java Island is documented in 16 publications, Sulawesi and Maluku eight publications, Kalimantan four publications, Lombok and Sumatra one publication, respectively, and Eastern Indonesia five publications (Figure 4). Most of the Indonesian research institutions are located in Java.

To develop research in other regions, research network has to be regularly created. Thus, acceleration on soil arthropod research in Indonesia can be implemented.

#### **Comparison with Other Countries**

##### *Malaysia*

The number of research on ecology of soil arthropods in Malaysia is higher than that in our country. Research on abundance changes of seasonal population and soil macrofauna has been conducted intensively (Kondoh *et al.*, 1980). They also compare differences of microhabitat, for example, in covered soils population of Diptera and Hymenoptera is higher than that in open soil, but population abundance of Thysanoptera is higher at the surface of open soil. Fluctuation of changes of seasonal population abundance and biomass in Pasoh do not reflect significant changes compared to research in Japan (Kondoh *et al.*, 1980). Research of taxonomy and behavior in Malaysia is not more than in Indonesia. Thus, both countries experience the same limitation.

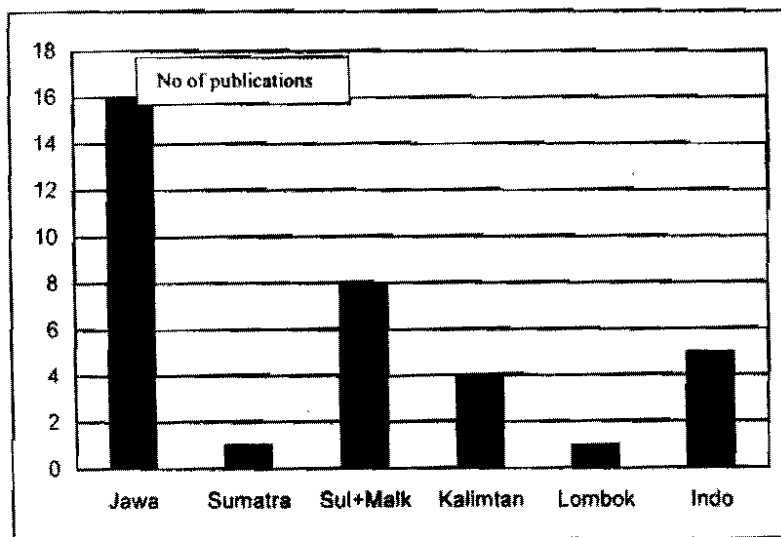


Figure 4. Research on soil arthropods in Indonesia based on regions.

### *Thailand*

Research works of some Japanese researchers (Takeda, 1981; Watanabe and Ruaysoongnern, 1984) have opened opportunity to investigate soil arthropods in Thailand. However, in general research advancement has not been satisfying. Research comparing abundance of Collembola in some types of ecosystems has been conducted; expressing that 23% of total soil arthropods is Collembola in forest ecosystems and 12% in agriculture ecosystems (Takeda, 1981). Population of Collembola in forest ecosystems is 4 times higher than that in agriculture ecosystems. Acari and Collembola predominate about 76% and 93%, respectively, from total micro arthropods in agriculture ecosystems (Takeda, 1981). Comparison the surface of cleaned and burned soil shows an increase of almost 50% of population abundance and biomass of macrofauna (Watanabe and Ruaysoongnern, 1984). At glance, number of research in Thailand is a little bit more than that in our country.

### *West Africa*

Research advancement of soil arthropods in West Africa is not so different from that in Indonesia. Works in ex- burnt soil shows degradation in number of species, individual and biomass. Mesofauna (Acari and Collembola) decrease about 45% and Lumbricidae and Collembola reduce to only 6% at high fire intensity. To return to the condition before fire takes 4 to 7 years for Collembola (Huhta *et al.*, 1969). Although forest fire continuously happens in Indonesia, intensive research to investigate the loss of soil fauna diversity fauna has never been carried out.

## **Present Status of Knowledge on Soil Arthropods in Indonesia**

### *Diversity*

Research on soil arthropods in Indonesia was initiated in Jawa Barat by Dammerman in 1925 and 1937, providing information on some species of soil arthropods, Mollusca, Vermes and Oligochaeta. Research was then continued by Indonesian researchers 20 years later (Soehardjan, 1957) and subsequently in the following 20 years (Adianto, 1979; Gunadi, *et al.*, 1992; Merciyanto *et al.*, 1997; Suhardjono, 1982; Suhardjono, 1985a; Suhardjono, 1985b; Suhardjono, 1997; Suhardjono, 1998; Soehardjono *et al.*, 1997). Unfortunately, all the research works have not been able to explain species diversity of soil arthropods in certain habitats in West Java. In general they only describe species based on rough morphological differences (morphospecies). Knowledge on diversity that we expect has not been realized.

The main constraint we face in investigating species diversity of soil arthropods is the absence of taxonomists. So far only certain families of Collembola that have been characterized (Deharveng and Suhardjono, 1994; Suhardjono, 1982; Suhardjono, 1989a; Suhardjono, 1989b; Yoshii and Suhardjono, 1992a; Yoshii and Suhardjono, 1992b). Reports on arthropods in agriculture ecosystems are even very scarce (Kalshoven, 1981).

### *Taxonomic ability*

Identification to the species level is absolutely demanded to explore diversity of soil arthropods. Unfortunately, knowledge on identification has not yet been available in our country. As a result, a lot of collections from arthropod researchers cannot be described precisely. Efforts to send samples abroad for identification are also almost impossible because of the high cost. This problem needs initiatives by high-level policy makers to build capacities of human resources with identification capability.

### *Soil fauna database*

Information transfer and acceleration are prerequisite to develop science. Information transfer can be accommodated through databases. Unfortunately, in Indonesia databases have not been available. In the United States of America, such databases have been built since 1991 by developing



software COMPESA (Computer Taxonomy and Ecology of Soil Animal), and in Europe ECOBASE/soil. Pusat Penelitian Biologi (Center for Biological Research), LIPI have published databases for diversities of all Indonesian flora and fauna called IBIS (Indonesian Biodiversity Information System) version 2.0.1, in which data input is still underway and NBIN (National Biodiversity Information Network). Both projects do not specialize for soil arthropods, but rather general. Therefore, a more specific database for diversity of soil biota is required for information transfer.

#### *Roles of arthropods*

Although knowledge on the roles of soil arthropods is obtainable from various studies in other countries, case studies of Indonesian experience have not been available. Therefore, it not surprising that we have not understood how to manage soil correctly from the view point of arthropod roles. This is very clear by the absence of proper soil management in Indonesia. The main constraint is lack of database as starting point.

#### **Development Program**

For the development of knowledge on soil arthropods in Indonesia, some strategic steps are needed. The steps are preceded by analysis of constraints and problems faced in the development program. Characterized problems have to be classified to determine priorities. These problem priorities that need to be solved include some examples below.

#### *Human resources*

Human resources represent early foundation for development of knowledge on soil arthropods. Human resources in general can be developed from various levels and certain authorities. In educational institutions they can be developed through students or young lecturers interested in conducting research on soil arthropods, while in research institutes by institute specification according to their mission to develop young researchers. These two society groups represent potencies that can be considered as key capital for scientific advancement.

These development efforts have to be designed based on proper steps and priorities. Things to be paid attention to in this step include short-term development, mid-term development, and long-term development. The steps should also indicate working priorities that must be completed. Below is an example.

1. Short-term (year 1 - 2)
  - Increase of quality and number of students
  - Formation of small groups in research or educational institutions to encourage human resource building
  - Development of joint activities to manage soil arthropods
  - Training of basic taxonomy and socialization of arthropod roles
2. Mid-term (year 2 - 4)
  - Development of field of studies dealing with soil biota (S2 and S3)
  - Formation of inter-institution working groups for human resource development
  - Establishment of joint activities between institutions (professional association)
  - Regular training on taxonomy and biology of soil arthropods for mid and high levels
3. Long-term (year 4-etc.)
  - Settlement of field of studies that specifically deal with problems of soil biota (taxonomy, ecology, behavior, physiology, etc.)
  - Establishment of working groups at national level to monitor human resource development
  - Development of national association of soil biota
  - Regular training on taxonomy and biology of soil arthropods at national level

### Research network

Research network is needed in developing knowledge on soil arthropods. Research network can be formed in a few steps, among others are as follows.

- Survey of all soil arthropod researchers in Indonesia
- Development of databases d having the same mission and vision nationally
- Expertation through formal institution or association
- Installment of motivator in research network formation

### Facilities

Facilities represent important components in developing knowledge on soil arthropods. Below are some aspects needed to be developed.

- Books are main keys in obtaining existing information. A major constraint faced by all students or researchers in their knowledge development is the limitation of journals and textbooks
- Lack of communication networks between soil arthropod researchers is also a serious constraint. One solution is formation of working groups that can be used for communications such as suggested above.
- Electronic media for information and book search have been used by some researchers. Therefore, development of electronic network needs to be formed to increase communication.

### Research program

Research aspects that require to be carried out include exploration of species diversity in various habitats. This can be conducted with cooperation between institutions having ecology and taxonomy capacities. Collectively they can develop short-term research programs in agriculture ecosystems, considering that land exploitation continues regardless of bad management methods, whereas information on agriculture ecosystem is still lacking. When elementary research (taxonomy) is finished, research on ecology and monitoring can be executed simultaneously.

### Conclusion

In developing knowledge on soil arthropods, some concrete steps must be taken. Firstly, development of knowledge on soil arthropods starts with installment of visions stipulating all lecturers and researchers the goals of the development to be reached together. Secondly, establishment of networks based on the visions we agree on. Thirdly, compilation of short-term, mid-term, and the long-term programs based on priorities agreed together.

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## **EFFECT OF LONGTERM CONSERVATION TILLAGES AND NITROGEN FERTILIZATION ON SOIL MESOFAUNA AND EARTHWORMS**

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### **Introduction**

Potency of dry land in Indonesia is reaching 56 million hectare, but only about 64 % representing productive land for agriculture. This is because the land is predominated by Ultisol and Oxisol types. These types of land represent marginal land needing additional actions for better productivity. Therefore, development of agriculture systems of emphasizing on land potency and environmental sustainability are required. Conservation tillages, including minimum tillage and no tillage, can be an alternative solution (Utomo, 1990).

Chemical and energy inputs at the intensive agriculture system should be reduced to increase economic resilience and environmental quality (Stinner and House, 1990). Due to the importance of biota in soil as an indicator of land quality and productivity, so that the knowledge on activity of soil biota is of vital importance.

For some years, soil biota have not yet gotten a lot of attention although it has been known that they play important roles in dynamic processes in agricultural ecosystem (Crossley *et al.*, 1989). Soil biota play important roles in improving soil organic matter through their role as early decomposers of organic litter. Collembola and micro-arthropodes play important roles in controlling decomposition process of organic matter (Dittmer and Schrader, 2000). Earthworms have an effect on the nature and characteristics of soil through their eating, casting and caving activities. Through these activities, earthworms can improve infiltration, aeration and stability of soil aggregates. In addition, earthworms casting has higher nutrition contents compared to the surrounding soil (Kladivko, 2003). Nevertheless, soil biota are very sensitive to disturbance caused by soil tillages.

Soil conservation tillage systems represent one of alternatives to reduce erosion and "take care of" soil, improve biodiversity and soil organic matters, and maintain crop productivity (Edwards *et al.*, 1993). In soil conservation tillage systems, crop rotation and herbicide use to depress weed growth, and also mulching are important.

Continuous use of herbicide in soil conservation tillage systems is considered to decrease non-target soil biota. Therefore, the herbicides used in soil conservation tillage systems should be easy to degradation in soil. Some research results showed that application of glyphosate herbicide of up to 21 seasons did not decrease population of arbuscular mycorrhizal fungi (AMF) (Yusnaini *et al.*, 1999), diversity of earthworms and mesofauna (Niswati *et al.*, 1997) and population of *Rhizobium* (Yusnaini *et al.*, 1997). Molla *et al.* (1987) mentioned that at normal dose, pesticides do not have an effect on soil mesofauna.

Earlier long-term research is needed as earlier investigations indicated that sufficient time is required before some soil conservation systems reached the balance of production, diversities of weed and soil biota, soil organic matter contents, and chemical properties of soil (Alamaras and Dowdy, 1985).

Therefore, studies of impact of long-term conservation tillage systems by using glyphosate, and nitrogen fertilization on population and activities of soil biota are worth pursuing.



## Materials and Methods

### Research Location

Experiments were conducted at the Politani, Unila experimental station, Hajimena, Bandar Lampung, Indonesia, representing location of long-term research on conservation tillages, started in 1987. Crop rotation applied was cereals (corn or upland rice)-legume (soybean, peanut, or green nut). Nitrogen fertilization was only applied to cereals.

Factorial treatments (3x3) in a completely randomized block design with 4 replications. The first factor was conservation tillage systems (intensive soil tillage-IT, minimum soil tillage-MT, and no soil tillage-NT), and the second factor was dose of N fertilization (without N-N0, 100 kg N ha<sup>-1</sup>-N1, and 200 kg N ha<sup>-1</sup>-N2). As control, herbicide-free soil with *Imperata cylindrica* vegetation was used.

At the intensive soil tillage treatment, soil was hoed and harrowed up to 25 cm, then litter and weed were cleaned. At the minimum soil tillage, soil was hoed, litter and weed were brought back to the soil as mulch. At the no-tillage treatment, soil was not mechanically manipulated at all except hole or slit for sowing the seeds, litter and weeds were used as mulch. Weeds were controlled using isoprophyl amine glyphosate with the doses of 7 L ha<sup>-1</sup>; 5 L ha<sup>-1</sup>; 3,5 L ha<sup>-1</sup> of Round Up, respectively, for the first, second, and third season, then for the following seasons the dose was 1,75 L ha<sup>-1</sup> of Round Up.

Nitrogen fertilization was applied in two steps, i.e. 1/3 dose was given 7 days after planting, and the rest was given just before corn flowering. TSP and KCl with doses of each 200 kg ha<sup>-1</sup> and 100 kg ha<sup>-1</sup>, respectively, were used as basal fertilizers. Corn varieties used were Pioneer-5 and Pioneer-12 with the planting distance of 20 x 75 cm<sup>2</sup>.

### Sampling

Soil sample was taken at the planting seasons of 21 and 29 on cornfield. Sample was taken in November 1996 (before application of herbicide and fertilizer before planting) and January 1997 (after application of herbicide and fertilizer before harvesting). At the end of 28<sup>th</sup> planting season, all plots of conservation tillage were tilled and soil samples were taken in January 2001 (before plowing), and May 2001 (after plowing).

In each experimental field, as many as 5 soil samples were taken randomly using a ring (5,5 cm diam., 6 cm height) for soil mesofauna, and a wooden box (25 x 25 cm<sup>2</sup>) for litter mesofauna and earthworms. Mesofauna samples were extracted with the modified method of *barlesse tulgreen* (Jackson and Raw, 1966). Population of earthworms was perceived with the method of direct enumeration (hand sorting method) (Anas, 1990).

For observation of soil chemical properties, as many as 3 soil samples were taken randomly from each experimental field, then bulk in composites. Soil chemical properties observed were pH (tetrameter), organic-C (Walkley and Black), and total-N (Kjeldhal) (Thom and Utomo, 1991).

Data were analyzed using analysis of variance and continued with multiple comparison of LSD at the 5% level. Diversity index of mesofauna at each experimental field was evaluated using Shannon diversity index (Odum, 1971).

## Results and Discussion

### Earthworms

Earthworms represent soil macrofauna whose existence in soil is of vital importance in improving soil quality. In general, earthworms play roles as early decomposers of organic matters, and in improving soil aggregates. Earthworm's existence in soil is influenced by soil microclimate. Earthworms grow and multiply in general at soil with pH 6-7, high organic matter contents, and humidity of 25% - 40%.

At planting season 21, experiment on conservation tillage systems indicated that population and biomass of earthworms before planting (November 1996) did not differ for both soil tillage and

nitrogen fertilization treatments, but showed significant differences before harvesting (January 1997) (Tables 1 and 2).

LSD test indicated that earthworm population and biomass before crop harvest were significantly different between conservation tillage (MT and NT) and intensive soil tillages (IT).

In Table 1 shown that population of earthworms before harvest in conservation tillages was higher than in natural conditions. This indicates that conservation tillage systems can improve soil micro environmental conditions, generating positive impacts to earthworm's existence. This can also be seen from the increase of soil organic matter contents (C-organic), total-N, pH and more supportive temperature for earthworm growth at conservation tillages (Table 5).

In general, earthworm genera found in all treatments were *Pheretima* and *Phantoscolax*. This indicates that both nitrogen fertilization and soil tillage treatments did not affect generic diversity of earthworms.

At the 29<sup>th</sup> season, before planting (before soil tillages), soil tillage treatments did not show significant difference in the biomass and population of earthworms, while after crop harvest (after soil tillages) earthworm population was influenced by soil tillage treatments. Earthworm population was highest at the no-tillage system, and differed from earthworm population at minimum and intensive tillage treatments. Less effect of conservation tillage on earthworm population before planting time was mainly due to after 28 planting seasons, the whole soils were plowed in both intensive soil tillage and conservation tillage treatments. Soil samples were taken before (January 2001) and after (May 2001) soil tillages.

Table 1. Effect of long-term conservation soil tillage systems and nitrogen fertilization on population of earthworms in cornfield

Treatment	Earthworm population (individuals m <sup>2</sup> )			
	21 <sup>st</sup> season		29 <sup>th</sup> season	
Soil tillage	Before planting	Before harvesting	Before planting	Before harvesting
Intensive tillage	149.33 a	177.33 a	88.87 a	69.33 a
Minimum tillage	282.67 a	636.08 b	87.11 a	72.89 a
No-tillage	194.57 a	455.75 b	100.22 a	101.33 b
N fertilizer (kg N ha <sup>-1</sup> )				
0	213.33 a	394.67 a	101.33 a	78.22 a
100	201.33 a	469.08 a	92.44 a	85.33 a
200	212.00 a	405.42 a	92.44 a	83.55 a
Control	362		84.16	

Note: At same column, numbers followed by same letters do not differ at 5% level of LSD test.

Non-significant difference of population and biomass of earthworms among treatments before planting was possibly caused by soil compaction of at the NT system. Compaction can inhibit earthworm proliferation. Moreover, this might also be caused by dry condition at the time of observation. The same results were obtained by Kladrilko (2003) who found that conservation and conventional tillages did not significantly affect earthworm population after 10 years of corn planting in the summer season. After crop harvest, however, NT treatment that had previously been plowed, improved soil structure, so that the earthworms can grow and strive better as shown by increase of earthworm population.

Compared to results of planting season 21, population and biomass of earthworms were of lower. This might possibly caused by of season 29 the lower organic matter contents as well as pH at planting season 29 compared to planting season 21 (Table 5). Earthworms grow and develop better at pH of about 6 and optimum at pH 7.

Table 2. Effect of long-term conservation soil tillage systems and nitrogen fertilization on biomass of earthworms in cornfield

Treatment	Earthworm biomass (g m <sup>-2</sup> )			
	21 <sup>st</sup> season		29 <sup>th</sup> season	
	Before planting	Before harvesting	Before planting	Before harvesting
Soil tillage				
Intensive tillage	2,99 a	1,71 a	0,42 a	0,39 a
Minimum tillage	8,99 a	28,78 b	0,41 a	0,38 a
No-tillage	5,38 a	14,22 b	0,46 a	0,44 a
N fertilizer (kg N ha <sup>-1</sup> )				
0	5,94 a	6,78 a	0,40 a	0,43 a
100	3,51 a	16,68 a	0,47 a	0,41 a
200	7,92 a	21,25 a	0,42 a	0,36 a
Control	10,64		1,50	

Note: At same column, numbers followed by same letters do not differ at 5% level of LSD test.

#### Soil mesofauna

The number of litter and soil mesofauna were not influenced by soil conservation systems and nitrogen fertilization, except the number of soil mesofauna before planting (November 1996) (Table 3). Diversity of litter and soil mesofauna were neither influenced by soil conservation systems and nitrogen fertilization, except diversity of litter mesofauna before harvest (January, 1997) (Table 4). The lowest diversity of litter mesofauna was obtained at the intensive soil tillage treatment, while diversity of litter mesofauna at the treatments of minimum tillage and no-tillage was not significantly different (Table 4).

Table 3. Effect of long-term conservation soil tillage systems and nitrogen fertilization on population of litter mesofauna and soil mesofauna in cornfield at the season 21

Treatment	Mesofauna litter (ind. m <sup>-2</sup> )		Soil mesofauna (ind. dm <sup>-3</sup> )	
	21 <sup>st</sup> season		29 <sup>th</sup> season	
	Before planting	Before harvesting	Before planting	Before harvesting
Soil tillage				
Intensive tillage	658,31 a	269,33 a	94,05 a	60,76 a
Minimum tillage	981,33 a	250,33 a	164,74 b	62,51 a
No-tillage	610,00 a	251,33 a	164,69 b	91,71 a
N fertilizer (kg N ha <sup>-1</sup> )				
0	838,67 a	194,66 a	137,84 ab	63,67 a
100	204,00 a	325,33 a	100,48 a	76,53 a
200	557,33 a	196,67 a	190,44 b	74,78 a
Control	149,28		84,16	

Note: At same column, numbers followed by same letters do not differ at 5% level of LSD test.

Table 4. Effect of long-term conservation soil tillage systems and nitrogen fertilization on diversity of litter mesofauna and soil mesofauna in cornfield at the season 21

Treatment	Diversity of litter mesofauna		Diversity of soil mesofauna	
	Before planting	Before harvesting	Before planting	Before harvesting
Soil tillage				
Intensive tillage	1,17 a	1,27 a	1,49 a	1,50 a
Minimum tillage	1,14 a	1,65 b	1,61 a	1,49 a
No-tillage	1,06 a	1,78 b	1,70 a	1,65 a
N fertilizer (kg N ha <sup>-1</sup> )				
0	1,16 a	1,53 a	1,57 a	1,54 a
100	0,93 a	1,53 a	1,66 a	1,49 a
200	1,27 a	1,64 a	1,57 a	1,62 a
Control	1,40		1,50	

Note: At same column, numbers followed by same letters do not differ at 5% level of LSD test.

Diversity index of litter mesofauna was influenced by soil conservation systems before crop harvest (Table 4). Lowest diversity (1,27) was found at the intensive soil tillage before harvest, which differed from diversity at the minimum soil tillage (1,65) and no-tillage (1,78). High diversity at the conservation tillage compared to intensive tillage was attributed to accumulation of organic matters on the surface of the soil. Organic matters at soil surface are food sources for soil mesofauna, resulting in higher diversity. This is related to the roles of the mesofauna as soil biota decomposing organic matters. This results are in line with results obtained by Sabatini *et al.* (1997) and Stiner *et al.* (1988) that Shanon-Wiever index diversity is higher at the minimum tillage than conventional tillage with shallow plough (0-25 cm) or deep plough (0-50 cm) systems. *Collembola* and *Acarina* are dominant mesofauna compared to others.

#### *Alteration of soil chemical properties*

Response of soil biota to ecosystem changes differed in each group of soil biota. Therefore, it is important to understand primary factors playing roles in influencing activity of soil biota. One of soil characteristics related to activity of soil biota is soil reaction (pH) and soil organic matter contents.

In Table 5 it is shown that in all of treatments, soil pH tends to decrease compared to natural vegetation, and this decrease is highest at the intensive soil tillage followed by fertilization. This pH decrease affects soil fauna. Earthworms are very sensitive to soil pH change. Although organic matters are abundantly available, at the pH of below 5 earthworms are difficult to grow and multiply (Yusnaini *et al.*, 1999).

Table 5. Alteration of some soil chemical and physical properties by application of long-term soil tillage techniques and nitrogen fertilization

Treatment	pH		Org.-C (%)	Tot.-N (%)	Temperature (°C)	
	H <sub>2</sub> O	KCl				
Planting season 21						
Control	6.20	5.80	1.60	0.20	-	-
IT NO	5.51	4.58	1.80	0.13	33.25	26.75
IT N1	5.31	4.37	1.83	0.14	34.00	26.83
IT N2	5.09	4.13	1.96	0.14	33.33	26.75
MT NO	5.81	4.85	2.90	0.15	33.25	25.66
MT N1	5.58	4.57	2.40	0.16	33.75	26.50
MT N2	5.31	4.31	2.35	0.14	32.75	27.00
NT NO	5.80	4.81	2.30	0.15	34.25	25.80
NT N1	5.46	4.56	2.25	0.14	33.25	26.50
NT N2	5.08	4.16	2.24	0.15	32.66	26.50
Planting season 29						
IT NO	4.99	4.55	1.52	0.09	27.67	27.00
IT N1	5.18	4.81	1.61	0.05	27.17	27.83
IT N2	5.08	4.69	1.52	0.09	27.83	27.17
MT NO	4.81	4.29	1.52	0.11	28.33	27.00
MT N1	4.95	4.65	1.76	0.08	26.83	26.67
MT N2	5.03	4.62	1.65	0.09	27.83	27.17
NT NO	4.6	4.29	1.68	0.08	26.33	26.83
NT N1	4.75	4.52	1.68	0.13	28.17	26.83
NT N2	4.72	4.42	1.78	0.10	27.69	27.50

Note: IT = intensive tillage, MT = minimum tillage, NT = No-tillage

Different from soil pH, at the season to 21, soil organic-C content sharply increased compared to control (natural vegetation), especially at treatments of minimum tillage and no-tillage. This increased organic matter content affects positively activity and existence of soil organisms. This is because organic matters are energy sources for soil organisms to grow and strive.

### Conclusion

Long-term conservation soil tillage systems and nitrogen fertilization give varied effects at each treatment and time of observation. In general, conservation tillages (MT and NT) increase the earthworm population, especially before harvest at the season 21 (January 1997). This is also true for population and diversity of soil mesofauna and litter mesofauna.

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## MICROBIAL POPULATION AND DIVERSITY OF FUNCTIONAL GROUPS OF SOIL MICROBES AT DIFFERENT SOIL ECOSYSTEMS IN INDONESIA

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### Introduction

Soil organisms either macro-, meso- or microbiota play a very important role in the functioning of soil ecosystems. Their role in soil organic matter decomposition, nutrient cycling, nutrients availability and nutrient uptake, soil physical properties, prevention of nutrient leaching and growth inhibition of plant pathogens are important. These services are important to the functioning of soil ecosystems as well as for sustainable management of agricultural ecosystems.

Decomposition rates of litter depend on litter quality and microbial and faunal population and their activities (Swift *et al.* 1979). Microbial decomposition of litter has been reported to be primary limited by nutrients such as P-availability in tropical forests and plantations (Duah-Yentumi *et al.*, 1998). On the other hand, litter comminution by macrofauna and microphytophagous mesofauna and grazing of meso- and microfauna on fungi and bacteria releases nutrients. Below ground interactions among soil fauna, microbes and roots influence the above ground herbivore systems (Scheu *et al.*, 1999; Scheu and Setälä, 2002).

Functional groups of soil microbes are the groups of microbes, which have a certain ability where other microbes do not. Some of the important functional groups of soil microbes are nitrogen fixing bacteria, phosphate solubilizing microbes, cellulolytic degrading microbes and non-pathogenic *Pseudomonas*. Symbiotic (*Rhizobium*) and non-symbiotic nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* undoubtedly have a significant contribution to the nitrogen input in natural ecosystems. This is also true for phosphate-solubilizing microbes which make insoluble phosphate becomes soluble. This soluble phosphate is available for plants and microbes. Other groups of microbes have ability to produce cellulase enzymes enabling them to degrade cellulosic materials. Population and the activities of these functional groups of microbes are affected by quantity and quality of the organic matter input into a certain natural ecosystems as well as by the environmental factors such as availability of nutrients and microclimate.

Many reports have concluded that conversion of primary forest to secondary forest, plantation, agriculture land and pasture has a large impact on above ground biodiversity, carbon storage in biomass and soil and may substantially alter carbon sink. This conversion of primary forest also change, the amount and quality of organic matter input to that particular ecosystems, alter the microclimate such as temperature, humidity and soil water content significantly. These changes undoubtedly affect the microbial composition and their activities in the soil.

The new type of ecosystems may play an outstanding role not only in respect to above ground biodiversity (Gentry, 1995) or water turnover (Stadtmüller, 1987) but also to carbon sequestration and storage (Soepadmo, 1993; Dixon *et al.*, 1994). However, knowledge about below ground biodiversity especially in the tropical rain forest is very limited. The largest uncertainty with respect to carbon turnover in the tropical rain forest yet exist in the below-ground compartment in particular for processes such as root production and decomposition and its control by soil organisms. Report from the first phase of SFB Project has shown that the species composition are markedly affected by forest disturbance but the extend of diversity change differs greatly among the systematic groups (STORMA, 2003).

The aims of this study were to determine the total microbial population, their activities and the diversity of the functional groups of soil microbes at different soil ecosystems.

## Materials and Methods

Soil samples were collected from Jambi (Sumatra), Sanggau (West Kalimantan) and Palolo (Palu, Central Sulawesi) as shown in the Table 1. In Jambi, soil samples were collected from five soil ecosystems at Permanent Research Station of PT IFA-BIOTROP, Pasirmayang, Muara Bungo District. In West Kalimantan, soil samples were taken from five soil ecosystems at TPTI PT Barito Pacific Indonesia, Sub-district Sekadau, District Sintang. In Central Sulawesi, soil samples were collected from six soil ecosystems at Palolo region, STORMA Project. In total, soil samples were collected from 16 soil ecosystems.

The soil samples were collected from the depth of 0-5 cm, 5- 10 cm and 10-15 cm or 0-5 cm and 5-15 cm. From each soil ecosystems three sampling lines, which are perpendicular to the direction of slope (distance between sampling lines was 100 m), were selected. From each sampling line, composite soil samples from each depth were collected. Ten sampling points along a 50 m sampling line with a distance of 5 meters each were collected and mixed. These mean that nine soil samples were collected from each soil ecosystems.

Table 1. Locations and soil ecosystems used for soil sampling

Soil ecosystems	Jambi, Sumatra	Sanggau, West Kalimantan	Palolo, Central Sulawesi
Primary forest	V	V	V
Secondary forest	V	V	V
Logged over forest	V	-	-
Forest garden	-	-	V
Agroforestry	-	-	V
Small holder rubber plantation	V	V	-
Maize field	-	-	V
Shifting cultivation	-	v	V
Cleared forest	V	-	-
<i>Imperata</i> grass land	-	V	-

Soil samples were transported directly to the laboratory and the microbiological parameters were determined. Soil samples were previously pre-incubated in the laboratory for 7 days at field capacity water content. The pre-incubated soil samples were used to determine the population and the number of functional groups of microbes using plate-counting methods. One-tenth soil solution series was used in three replicates.

The microbiological parameters determined were the total number of propagules (Nutrient Agar) and number of fungi (Martin Agar). Microbial activity (soil respiration) was determined by using jar method. Functional groups of soil microbes were determined by using plate counting method on selective medium. Ashby medium was used to quantify the number of *Azotobacter*. Most probable Number (MPN) technique was used to determine the number of *Azospirillum* using Nitrogen Free Medium (NFB). Pikovskaya medium was used to count the number of phosphate solubilizing microbes. King's Medium B was used to determine the number of non-pathogenic *Pseudomonas* and Carboxyl-Methyl-Cellulose (CMC) medium was used to determine the number of cellulolytic microbes. The number of ammonium oxidizers and nitrite oxidizers was determined by using MPN methods and the number of denitrifiers was determined using MPN methods.

## Results and Discussions

Total number of propagules at 0-5 cm soil layer was significantly higher than that at the deeper layers (Figure 1). In Jambi and Palu (Central Sulawesi), the difference between the total number of propagules of the 0-5 cm layer and the deeper layers was much higher than in West Kalimantan. Almost in all soil ecosystems, the total number of propagules in the deeper layers was only about 25% or less than that in the 0-5 cm layer. The difference in the total number of propagules among the types of soil ecosystems at a certain region was also very obvious. In Jambi, the total number of propagules in secondary forest, logged over forest and clear-cut forest (1995) was about 60 to 80% of the total number of propagules in the primary forest. However, the total number of propagules in rubber plantation was much lower than in the other soil ecosystems. In the rubber plantation, the total number of propagules was only about 15% of the total number of propagules in the primary forest. In West Kalimantan, the same situation was also observed. The total number of propagules in rubber plantation was the lowest among the types of soil ecosystems studied. This was due to the differences in quality, quantity of the organic matter input and other environmental factors to those soil ecosystems. In the primary forest or secondary forest, input of organic matters was more and more heterogeneous than input of organic matter in the rubber plantation. More over, temperature, soil moisture and other factors were more favorable for microbes to grow in the primary forest than in the rubber plantation. In rubber soil ecosystems, soil organic matter input was more homogenous and the quantity was less than in the other soil ecosystems.

The number of colony forming units of fungi showed the same trend as for the total number of propagules (Figure 2). The number of fungi in the 0-5 cm layer varied from  $10^3$  to  $10^5$  cfu per gram of soil. The number of fungi in the 0-5 layer was higher than the number of fungi in the deeper layers. The number of fungi in 10-15 cm layer was about 20– 40% of the 0-5 cm layer in Jambi and West Kalimantan, but in Palu, the number of fungi in the deeper layer was much lower than in the 0-5 cm layer. In Jambi, the lowest number of fungi was observed in clear-cut forest. In Palu, the lowest number of fungi was observed in the maize field and agroforestry ecosystems.

In most cases, the population of microbes correlates positively with their activities (soil respiration). The higher the population in a certain soil ecosystem, the higher the soil microbial respiration. This means that the microbes in this soil ecosystem used more oksigen for their activities and produce more carbon dioxide (Figure 3). However, data of soil microbial respiration in three regions showed different figure. In Jambi, soil microbial respiration in primary forest was not the highest among the types of soil ecosystems studied. The soil microbial respiration in the deeper layers was not always lower than soil microbial respiration of the 0-5 cm layer. In West Kalimantan, the difference in soil microbial respiration among the soil ecosystems and among the layers was much higher. Soil microbial respiration in the *Imperata* grassland was less than 50% of soil microbial respiration in the primary forest. In Palu, soil microbial respiration was more or less the same in the different types of soil ecosystems. The difference in soil microbial respiration was significant with soil depth.

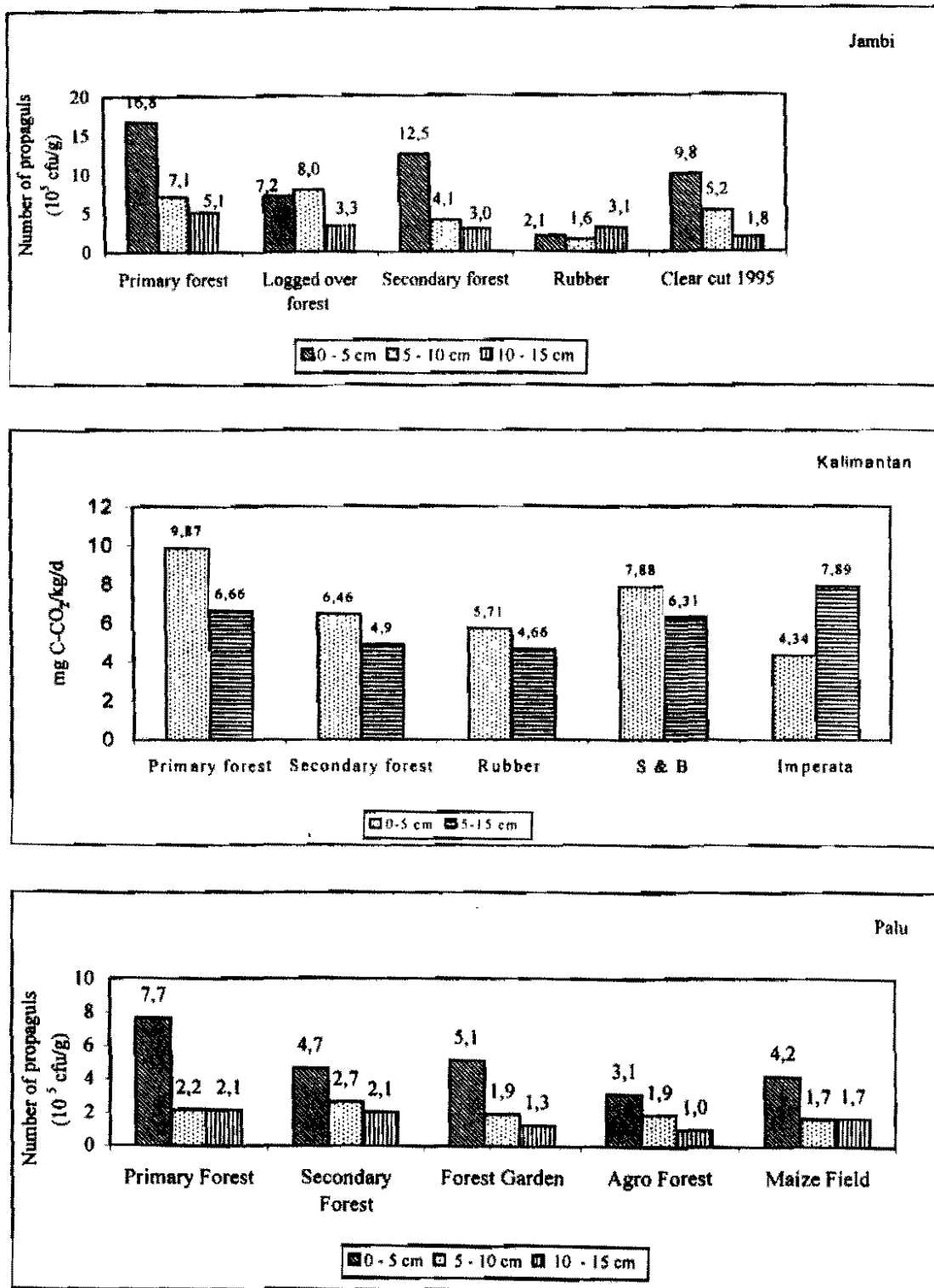


Figure 1. Total number of propagules at different types of soil ecosystems in Jambi, West Kalimantan and Central Sulawesi.

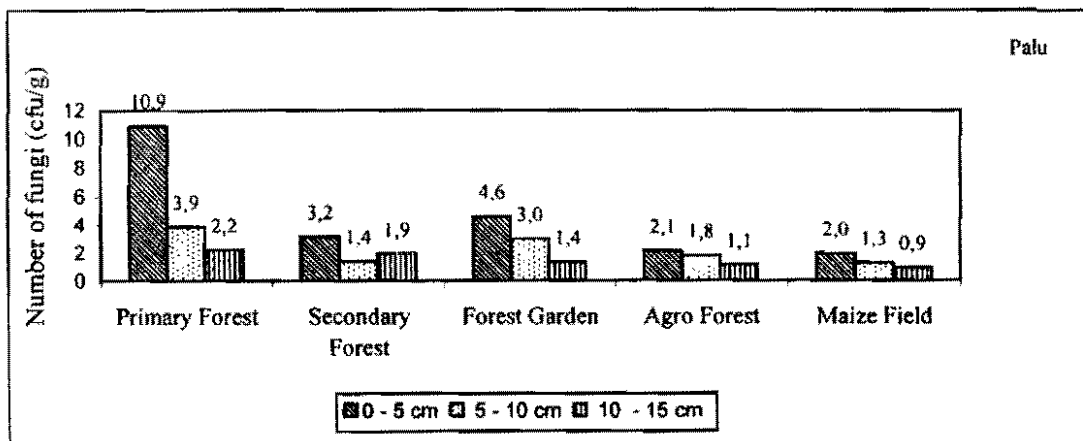
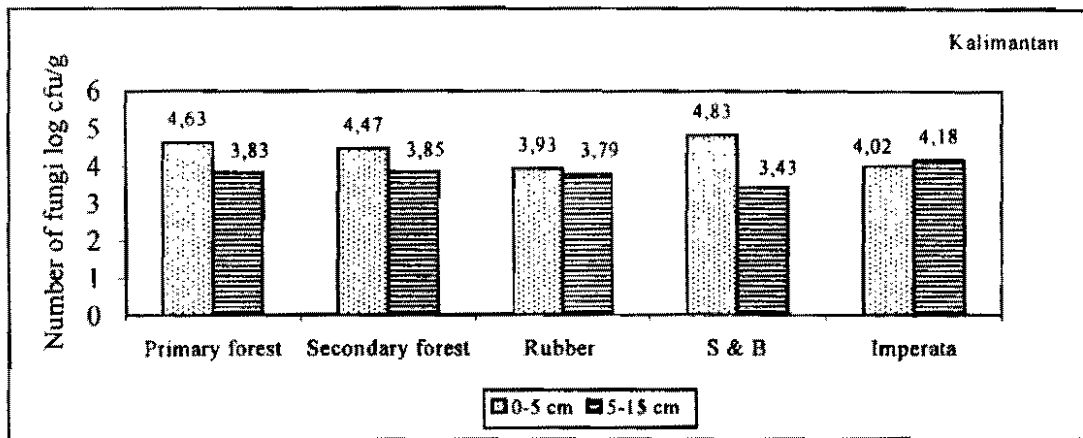
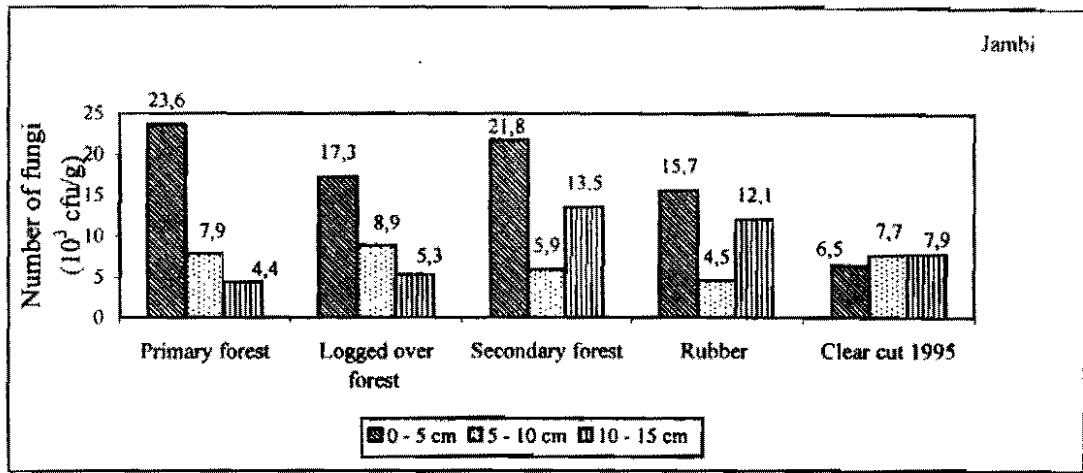


Figure 2. Total number of fungi at different types of soil ecosystems in Jambi, West Kalimantan and Central Sulawesi.

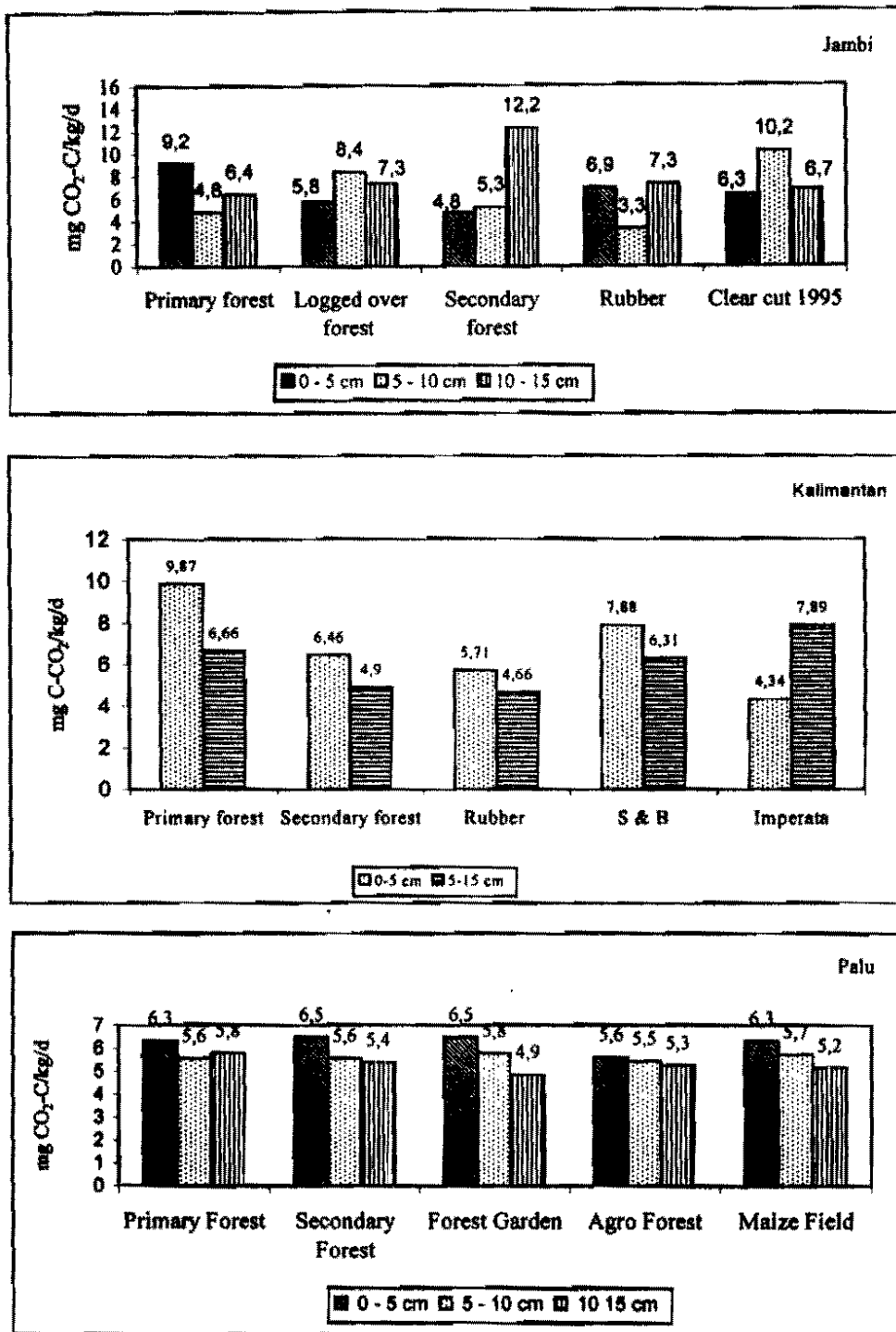


Figure 3. Soil respiration at different types of soil ecosystems in Jambi, West Kalimantan and Central Sulawesi.

The population of nitrogen fixer *Azotobacter*, *Azospirillum*, the number of phosphate solubilizing soil microbes (PSM), number of non pathogenic *Pseudomonas*, and the number of cellulolytic microbes were only determined from the soil samples taken from West Kalimantan (Figure 4, 5, 6, 7 and 8). The number of *Azotobacter* was almost the same at all types of soil ecosystems. The difference in the number of *Azotobacter* was only observed within the first 0-5 cm compared to the deeper layer 5-15 cm. The population of *Azotobacter* in the 5-15 cm layer was much lower than the population of *Azotobacter* in the 0-5 cm layer. Very interesting result was also observed in the number of nitrogen fixer *Azospirillum*. In the primary forest and secondary forest, no *Azospirillum* were detected with the technique used. However, the number of *Azospirillum* in rubber plantation, slash and burn agricultural systems as well as in *Imperata* grassland, the number of *Azospirillum* was in the order of  $10^3$  to  $10^4$  sel per gram soil. This means that in these three soil ecosystems, *Azospirillum* may play important role in supplying nitrogen to these soil ecosystems. On the other hand, *Azospirillum* plays unimportant role in supplying nitrogen to primary forest as well as to secondary forest soil ecosystems. This also means that other mechanisms of supplying nitrogen to this particular soil ecosystem might occur.

Phosphate solubilizing microbes either bacteria or fungi are the main phosphate solubilizing microbes in the soil. These microbes were present in the upper layer as well as in the deeper layer. There was a little variation in the number of this microbial group in different types of soil ecosystem. The number of non-pathogenic *Pseudomonas* was low in the secondary forest compared to the other soil ecosystems. The obtained data could not explain why this group of microbes was less in secondary forest compared to other soil ecosystems. Cellulolytic microbes play very important role in decomposition process. The number of cellulolytic microbes was very low in the slash and burn soil ecosystems either in the 0-5 cm or in the 5-15 cm layer while in other soil ecosystems, the number of cellulolytic microbes was similar.

Ammonium oxidizers in Jambi or in Palu did show the same pattern. It was less in the primary forest and much less in rubber plantation or maize field but it was high in secondary forest and logged over forest (Jambi) or forest garden (Palu) (Figure 9). The number of ammonium oxidizers in these types of soil ecosystems showed the same pattern that the number of ammonium oxidizer in the 0-5 cm soil layer was much higher than the deeper layer. In Figure 10, the number of nitrite oxidizers in all soil ecosystems in Jambi and Palu were about the same, ranging from around  $1 \times 10^4$  to  $2 \times 10^4$  cfu per gram of soil. The number of denitrifier in Jambi and Palu (Figure 11) showed different results. In Jambi, the number of denitrifier in primary forest was less than in the number of denitrifier in rubber plantation as well as in the clear-cut forest. Moreover, the number of denitrifier in the 10-15 cm was much higher than in 0-5 cm soil layer in three of five types of soil ecosystems. Contrary to this, in Palu, the number of denitrifiers in the primary forest was significantly higher than that in other soil ecosystems and the number of denitrifier at 0-5 cm in all soil ecosystems was constantly higher than in the deeper soil layers.

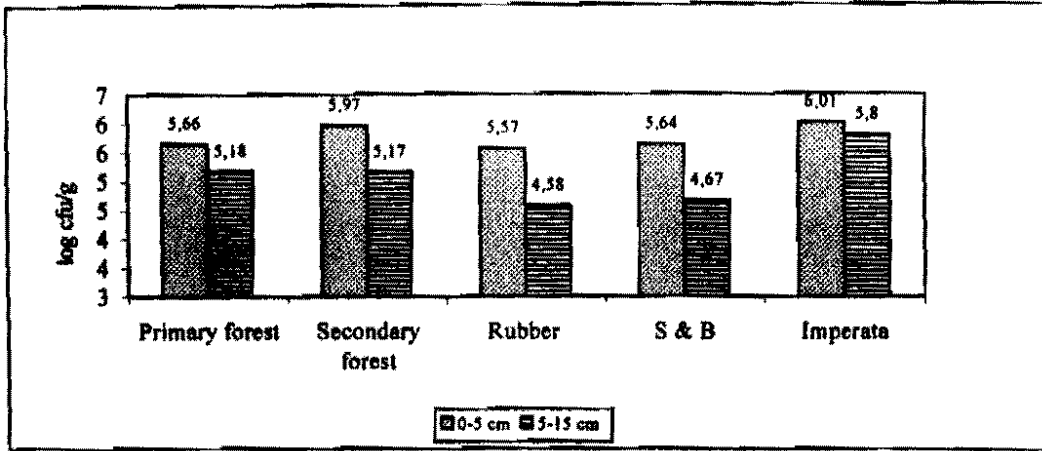


Figure 4. Population of *Azotobacter* at different types of soil ecosystems in Sanggau West Kalimantan

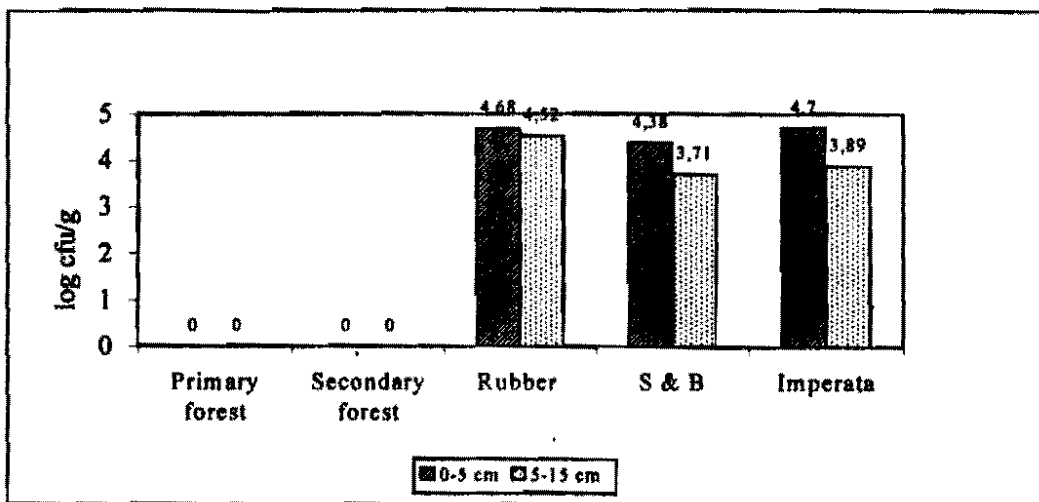


Figure 5. Population of *Azospirillum* at different types of soil ecosystems in Sanggau West Kalimantan



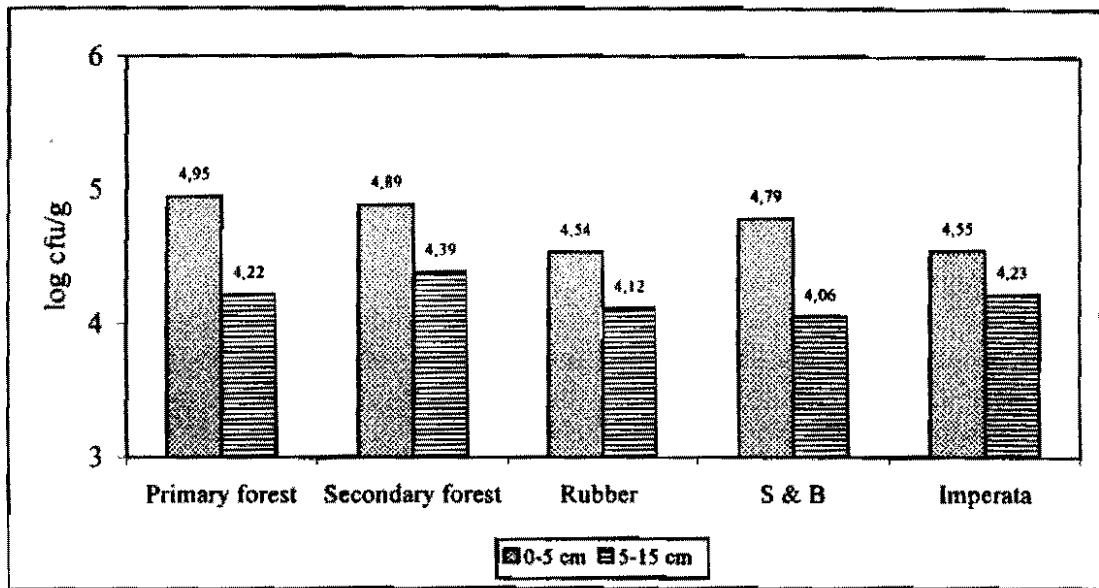


Figure 6. Population of phosphate solubilizing microorganisms at different types of soil ecosystems in Sanggau West Kalimantan.

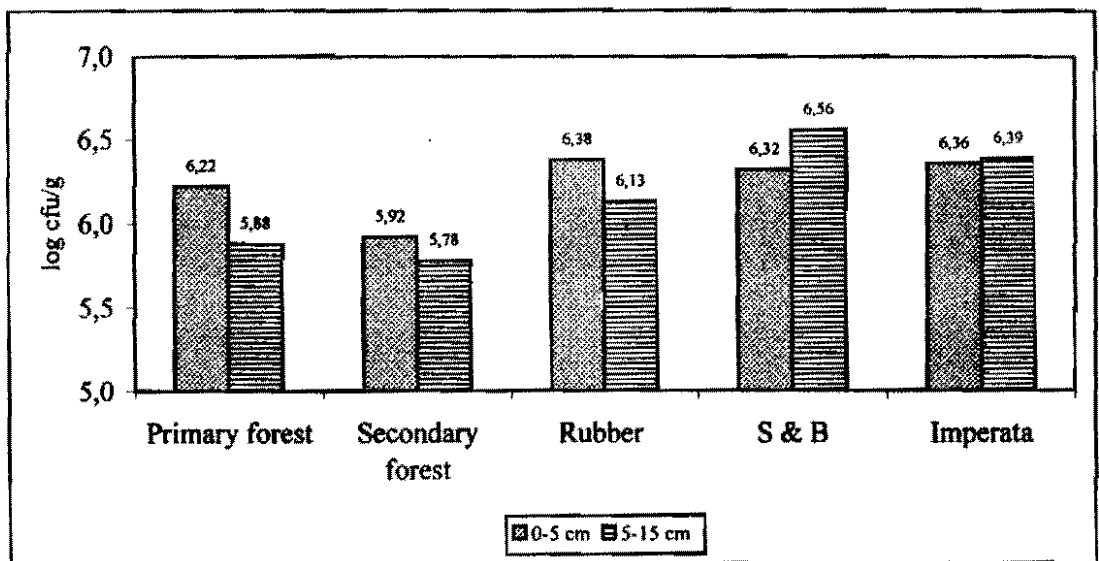


Figure 7. Population of non-pathogenic *Pseudomonas* at different types of soil ecosystems in Sanggau West Kalimantan.

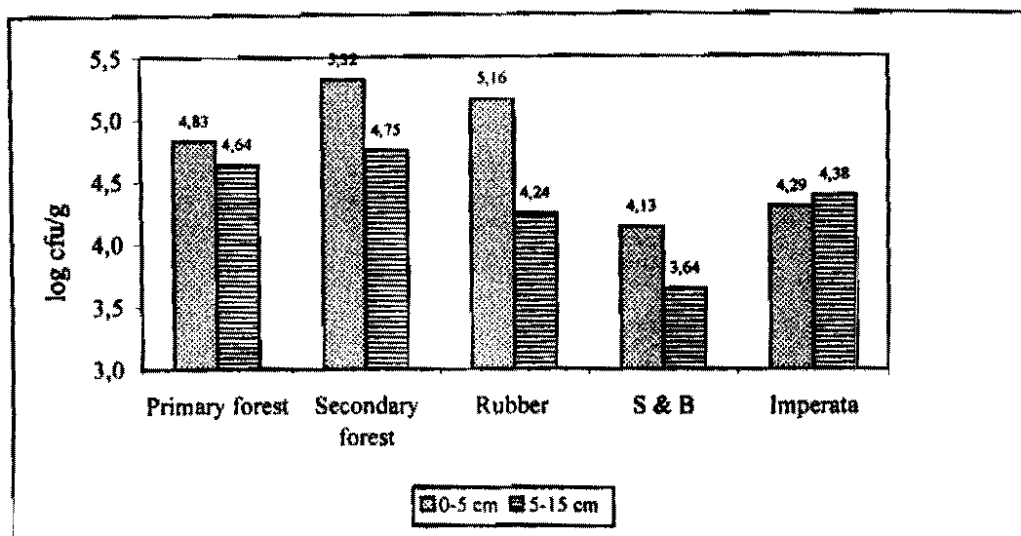


Figure 8. Population of cellulolytic microbes at different types of soil ecosystems in Sanggau West Kalimantan.

### Conclusions

Conversion of primary forest to secondary forest, rubber plantation, agriculture land, forest garden reduces microbial population and their activities. Population of functional groups of soil microbes varies significantly among soil ecosystems. A certain functional group of soil microbes may be missing at a certain soil ecosystem.

### Aknowledgements

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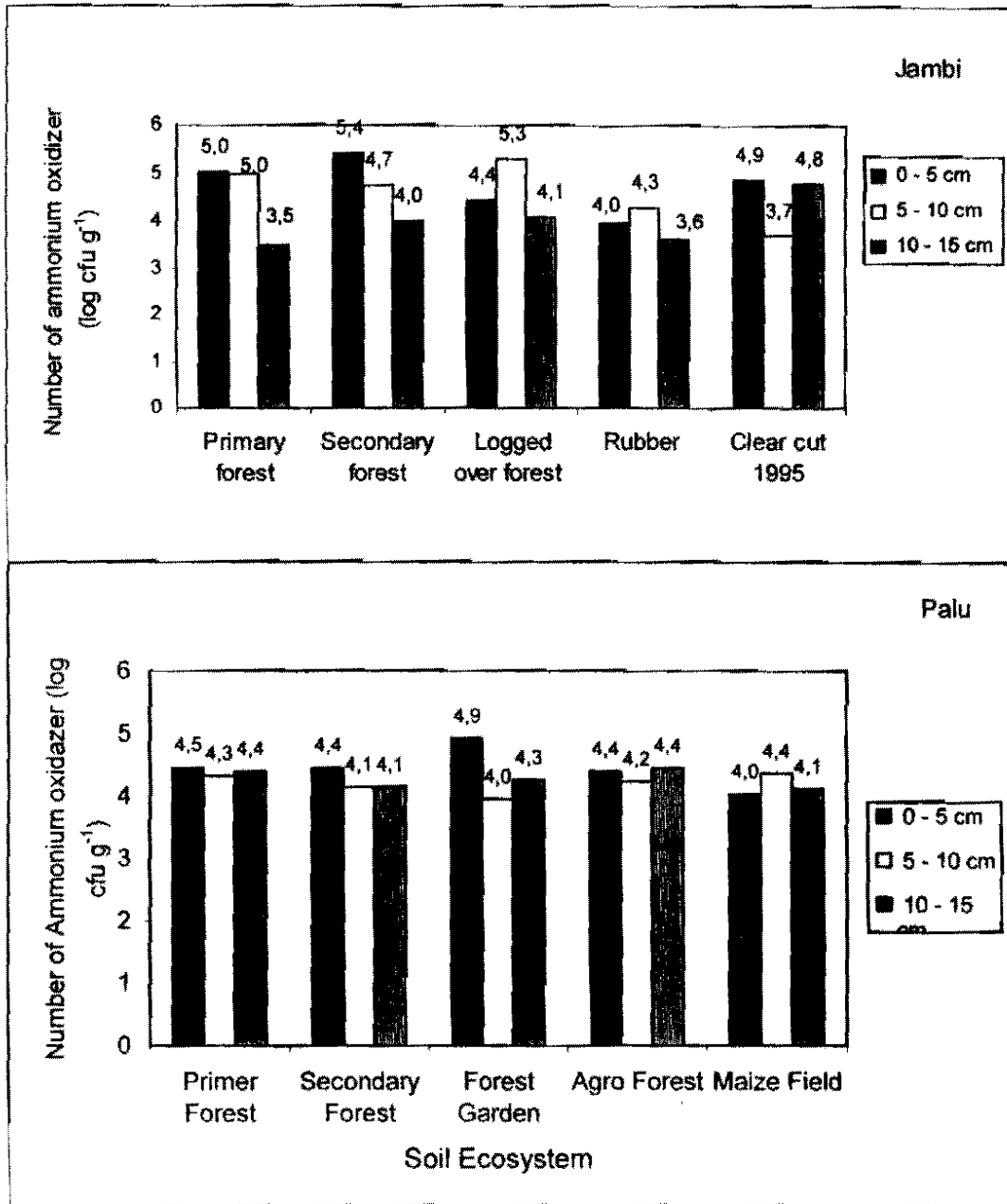


Figure 9. Ammonium oxidizer at different types of soil ecosystems in Jambi and Central Sulawesi

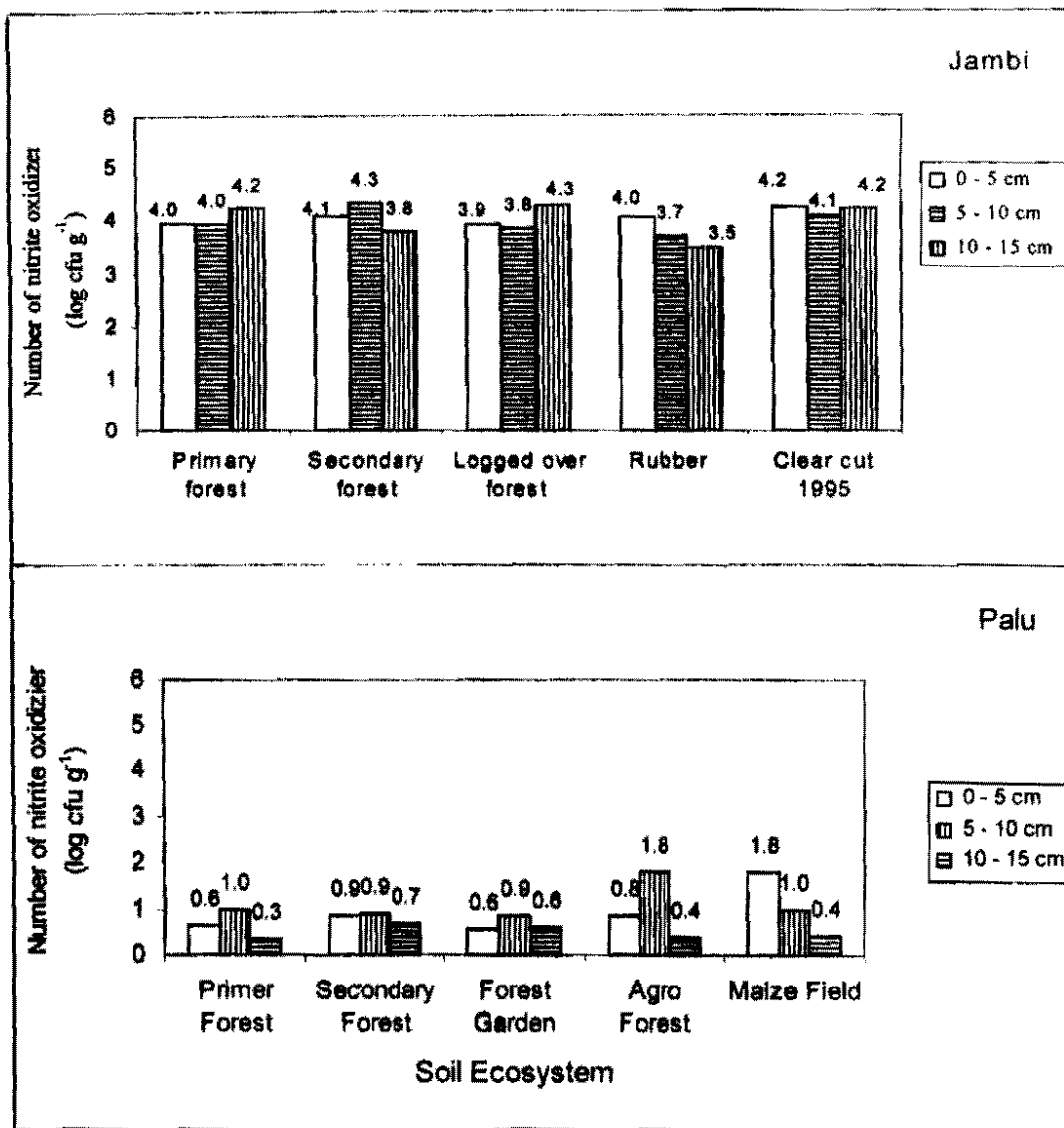


Figure 10. Nitrite oxidizer at different types of soil ecosystems in Jambi and Central Sulawesi

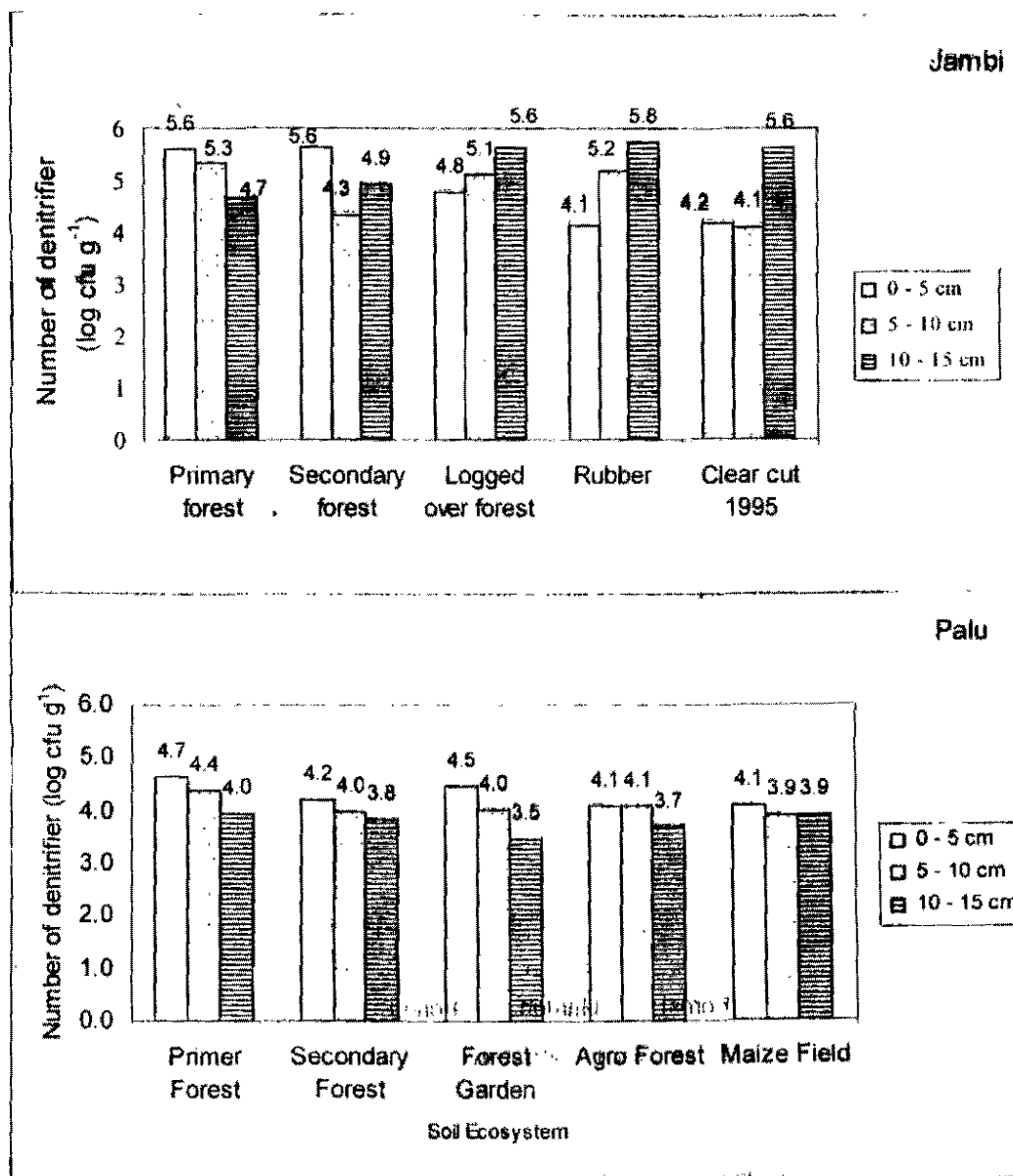
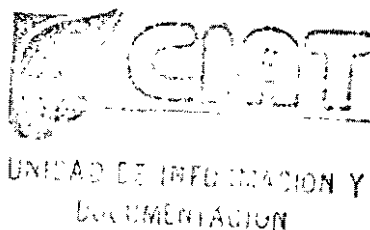


Figure 11. Denitrifier at different types of soil ecosystems in Jambi and Central Sulawesi

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## PUTATIVE ECTOMYCORRHIZAL FUNGI AT THE SUNGAI WAIN PROTECTION FOREST, EAST KALIMANTAN

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### Introduction

Trees in the mixed Dipterocarp forest, a common forest type in Kalimantan, are well associated with ectomycorrhizal (ECM) fungi. The fungus has a mutualistic symbiosis with the roots of Dipterocarps. Within the relationship, the fungus takes up nutrient and water from the soil and transports them to the tree roots. In return, it receives sugar compounds from its host roots. Ectomycorrhizal associations are capable to prevent plant root diseases and increase drought resistance (Harley, 1972). Furthermore, ECM fungi play an important role in the tropical rain forest nutrient recycling (Smits, 1985).

Inventories of the ECM fungi in an undisturbed forest of East Kalimantan were studied and reported (Ogawa, 1992; Yasman, 1993; Smits, 1994). Over the six years observation period, about 172 species of putative ECM fungi were revealed to have association with 23 Dipterocarps, in which species of *Amanita*, *Boletus* and *Russula* act as the dominant fungi (Yasman, 1993). However, the correlation of the ECM fungi with the roots of the host plant was not discussed.

Forest fires decrease significant amounts of ECM fungi population and formation (Amaranthus, 1992; Nurjanto and Suhardi, 2001). Until recently, description of the ECM community species composition was almost exclusively based on sporocarp inventories. It was assumed that sporocarp production reflected the relative abundance or importance of the species in question in the soil (Dahlberg *et al.*, 1997; Yasman, 1993). In the year of 1998, some parts of the Sungai Wain protection forest were damaged by fire covering an area of 10.024 ha, and thus accounting for ca. 65 percent of the total protection forest area. That was the first fire incident reported (Russon and Susilo, 1999) causing the area to be severely burnt (Hoffman, 1999). The objective of the study is to identify the dominant ECM fungi in the unburnt and burnt parts of the Sungai Wain protection forest observed two years after the fire, based on the analysis of sporophores found on forest floor.

### Materials and Methods

#### *Study site*

The study was conducted in a mixed dipterocarp forest (MDF) at the Sungai Wain protection forest in East Kalimantan (1°1' – 1°14' and 119°48' – 116°58'), about 15 km northeast of Balikpapan. The MDF is located on lowland at an altitude of 150 m above sea level (Priadjati, 1999). The area has a flat to undulating topography. The climate is very humid (climate type A) with Q = 7.0 % and average annual precipitation of 2230 mm (Schmidt and Ferguson, 1951). Observations were conducted in six permanent established sample plots representing the burnt and unburnt sites. Each site consisted of three permanent sample plots of to 300 m x 20 m consisting of three rows of belt transects of 100 m x 20 m.

#### *Surveys of sporocarps*

Sporocarp survey was carried out in the study plot for four weeks from mid January to February 2000. The number of sporocarps of each potential ECM species was recorded. Identification of the fungi was based on the characteristics of the sporocarps (macroscopic characteristics) observed using the identification key prepared by Smits (1994). The species diversity of the ECM fungi in the burnt and unburnt sites was then determined.

**Results and Discussion**

The quantitative description analysis of the putative ECM fungi found in the unburnt dipterocarp forest is shown in Table 1. No fruiting bodies of ECM fungi were recorded in the burnt forest area. The result of the analysis of the correlation between assumed root system extent and occurrence of putative ECM sporocarps (Figure 1) is presented in Table 2.

During the four weeks observation time, thirty two sporocarps of fungi suspected to form ECM were encountered within the unburnt site containing 11 species and 4 families of Basidiomycetes, of which nine species belong to order Agaricales (genus *Amanita* and *Russula*) and two species belong to order Aphyllophorales (genus *Cantharellus* and *Ramaria*). The most common genus is *Russula* and the dominant species is *R. lilacea*. *Dipterocarpus tempehes* and *D. confertus* were suspected as the host trees of *R. lilacea*. Information on the identity and diversity of ECM fungi is necessary to assist further understanding of the relationship between mycorrhizal fungi and forest function (Lee, 1998).

Table 1. Quantitative description of the sporocarps of fungi suspected to form ECM in the unburnt dipterocarp forest

Species of fungi	Family	1 <sup>1</sup>	2 <sup>1</sup>	3 <sup>1</sup>	4 <sup>1</sup>
<i>Amanita tjibodensis</i>	Amanitae	1	3.13	6.25	9.38
<i>Amanita sychnopyramis</i>	Amanitae	3	9.38	18.75	28.13
<i>Amanita</i> sp.1	Amanitae	1	3.13	6.25	9.38
<i>Russula eburneoareolata</i>	Russulaceae	3	9.38	6.25	15.63
<i>Russula japonica</i>	Russulaceae	2	6.25	12.50	18.75
<i>Russula lilacea</i>	Russulaceae	4	12.50	18.75	31.25
<i>Russula</i> sp.1	Russulaceae	3	9.38	6.25	15.63
<i>Russula</i> sp.2	Russulaceae	5	15.63	6.25	21.88
<i>Russula</i> sp.3	Russulaceae	1	3.13	6.25	9.38
<i>Cantharellus</i> sp.	Cantharellaceae	6	18.75	6.25	25.00
<i>Ramaria</i> sp.	Clavulinaceae	3	9.38	6.25	15.63

1=Number of sporocarps, 2=Relative of frequency, 3=Relative Dominance, 4=Important Value Index.

Table 2. Sporocarp of putative ECM fungus found near dipterocarp species

Putative ECM Fungi	Tree species <sup>1</sup>					
	1	2	3	4	5	6
<i>Amanita tjibodensis</i> Boedijn				*		
<i>Amanita sychnopyramis</i> Corner & Bas		*		*	*	
<i>Amanita</i> sp.1						*
<i>Russula eburneoareolata</i> Hongo	*	*				
<i>Russula japonica</i> Hongo	*		*			
<i>Russula lilacea</i> Quel.	*	*				
<i>Russula</i> sp.1		*				
<i>Russula</i> sp.2				*		
<i>Russula</i> sp.3				*		
<i>Cantharellus</i> sp.			*			
<i>Ramaria</i> sp.					*	

<sup>1</sup>1=*Dipterocarpus tempehes*, 2=*D. confertus*, 3=*Shorea smithiana*, 4=*S. ovalis*, 5=*S. laevis*, 6=*S. lamellate*.



Sporocarps of Basidiomycetes ECM fungi are reproductive bodies. Observation in the unburnt forest (plot #7), suggested that there was a direct relationship between *Russula* sp. 3 and the roots of *S. ovalis*. ECM on the root tips of *S. ovalis* has white color. Similar relation and color of ECM were also found between the root tips of *S. ovalis* (plot #13) and fungus *A. sychnopyramis*. It is the fact that different fungi may morphologically form different ECM on the root system of a single plant. Although some ECM fungi show some host specificity at the host genus level, most ECM fungi generally have broad host ranges (Lee, 1998). It is shown that *A. sychnopyramis* is a broad host range ECM fungi. Table 2 provides an overview of the associations of the putative ECM fungi and 6 dipterocarp species, which were suspected as the host trees for ECM.

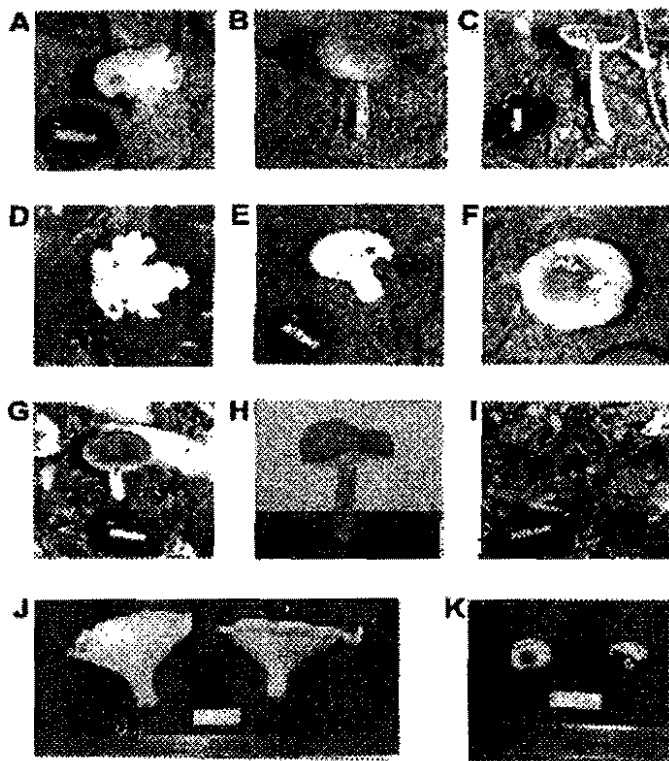


Figure 1. Sporocarps of fungi suspected to form ectomycorrhizae in unburnt forest area. A. *A. tjibodensis*; B. *A. sychnopyramis*; C. *Amanita* sp.1; D. *Cantharellus* sp.; E. *R. eburneocareolata*; F. *R. lilacea*; G. *Russula* sp.2; H. *Russula* sp.3; I. *Ramaria* sp.; J. *R. japonica*; K. *Russula* sp.1.

The fire occurring in the Sungai Wain protection forest in 1998 changed the composition of sporocarps of fungi suspected to form ECM on dipterocarps. It is predicted that the fire resulted in unsuitable microclimate conditions for the formation of sporocarps in the burnt forest. Microclimatic factors (*i.e.* air and soil temperature, relative humidity and solar radiation) in the unburnt site were found to be stable, whereas those in the burnt site were unstable. The solar radiation in the burnt site ranged from 93.47 to 826.96  $\mu\text{mol cm}^{-2} \text{s}^{-1}$  which was significantly higher compared to that in the unburnt site, which ranged from 19.74 to 269.53  $\mu\text{mol cm}^{-2} \text{s}^{-1}$  (Tata, 2001).

The decline of soil organic matter in the soil forms another possible explanation. Humus and debris, which together cover the upper ground layer of the soil, may prevent the decline of the soil

humidity and act as the nutrient supplier for fungi. It was observed that there was a limited amount of humus and debris in the burnt site. Soil organic content in the unburnt forest area was 6.31 %, whereas in the burnt forest was 5.78 % (Iriansyah *et al.*, 1999). Litter on forest floor may have a significant role in sporocarp formation, due to its function in providing nutrients for fungal development as well as preventing humidity of soil. Supriyanto and Setiawan (1995) stated that removing litters from forest floor inhibited the formation of *Scleroderma columnare* sporocarps on Dipterocarp stands, although microclimate conditions (*i.e.* light intensity, soil pH and relative humidity of soil) were suitable for sporocarp formation. Environmental factors, such as lack of soil organic matter and the high intensity and fluctuation of solar radiation, were suspected to inhibit the sporocarps formation.

Although there was no sporocarp found in the burnt site, the mycorrhizal potential inoculum in the soil was quite good. Different species of ECM fungi were able to survive in the burnt forest, as they were found in different colors, different hyphal diameter and clamp connection (Tata *et al.*, 2002).

### Conclusion

This research was conducted to study the diversity of ectomycorrhizal (ECM) fungi at the Sungai Wain protection forest, East Kalimantan. Surveys on the numbers of sporocarps and species of ECM fungi were carried out in two locations, *i.e.* burnt and unburnt forest area. Eleven species of putative ECM fungi were found in the unburnt forest area, while no sporocarps of ECM fungi were encountered on the forest floor, two years after the fire.

### Acknowledgment

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## OCCURRENCE OF NODULATION IN LEGUME SPECIES IN INDONESIA

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### **Introduction**

The family Leguminosae is distributed worldwide. The number of species of the family is estimated 16,000 to, 19,000 covering about 750 genera (Allen and Allen, 1981). Economically it is second to the grasses, Gramineae. In Indonesia the legumes are one of the most important families. Many legume species can be used as energy sources, timbers, foods, forages, ornamentals, medicinal plants, green manures, erosion control plants, fibers and botanical insecticides. Various species can grow well in different land use systems under different environments from lowlands to highlands, well adapted to a wide range of soil fertility. The ecological uniqueness of legumes centers on the nodules of their root systems.

Nodulation indicates the ability of nodule bacteria to infect plant root systems. For more than 100 years it has been established that root nodules bear a causal relationship to the fixation of free nitrogen. In the earlier time some scientists considered the occurrence of nodulation as an attribute to the Leguminosae. In fact, the ability to nodulate seems to be consistently absent within particular groups of the family. Therefore, it is the aim of the paper to review literatures on nodulation of legumes grown in Indonesia

### **Nodule Formation**

The absence of nodules does not necessarily indicate inability on the part of a plant to live symbiotically with nodule bacteria. There are two kinds of nodule-forming inability: 1) inability due to unfavorable temporary situations, 2) inherent inability to form nodules. Allen and Allen (1981) proposed four common explanations for the absence of nodules, i.e. 1) shedding of nodules due to drought, flooding, or clipping the plant's foliage, 2) unfavorable environmental conditions such as soil type and soil pH, temperature extremes, and insufficient solar radiation, 3) the absence of compatible rhizobia in the rhizosphere obviously precludes infection, 4) resistant or non-invasive lines resulting from plant breeding and selection.

Inherent inability to form nodules can be caused by the following factors: 1) the presence of physical and morphological barriers to invasion, such as wiry, dark colored rootlets and sparse thick-walled root hairs which are obvious macroscopically, on most of the non-nodulating species, 2) the presence of cell constituents containing tannins, flavonoids, quinines, or other phenolic compounds which exert an antibiotic and physicochemical restriction to rhizobial invasion and growth, 3) the absence of lectins, phytohemagglutinins, or essential enzymes from the root hairs, thereby accounting for the failure in recognition or binding between the rhizobia and the root surface, 4) the absence of disomatic cells in the cortical root tissue, 5) a relationship between the chromosomal number and the presence of nodules.

### **Occurrence of Nodulation In Legume Species**

The legume species grown in Indonesia are listed in Table 1, 2, and 3., mainly based on the works of Keuchenius (1924), Heyne (1927), Allen and Allen (1981), and Nielsen (1992) only for Table 2. There are 26 genera and 79 species listed in the subfamily Caesalpinioideae, 20 genera and 137 species in the subfamily Mimosoideae, and 66 genera and 166 species in the subfamily Papilionoideae.

Conflicting reports are observed in five species of Caesalpinioideae, i.e. certain species of the genera *Amherstia*, *Cassia*, and *Intsia* were reported nodulated and non-nodulated (Table 1). This phenomena can be due to two main groups of factors as mentioned by Allen and Allen (1981), i.e. inability due to unfavourable temporary situations, and inherent inability to form nodules.

In Caesalpinioideae, 15% of the genera and 14% of the species were found to be nodulated. This subfamily is the least nodulated one among three subfamilies. In Mimosoideae, the ability to nodulate was 55% at the genera level and 28% at the species level, whereas in Papilionoideae 77% and 73%, respectively. The compilation of the data shows that the ability to nodulate is about 51% in the subfamily Caesalpinioideae, 69% in the subfamily Mimosoideae, and 23% in the subfamily Papilionoideae still unknown.

Table 1. Nodulation in the subfamily Caesalpinioideae

Legume species	Nodulation and references
<i>Acrocropus fraxinifolius</i> W. & A.	Nr
<i>Amherstia nobilis</i> Wall.	+ Wright cit. Allen & Allen, 1981; - Lim, 1977
<i>Bauhinia acuminata</i> L.	- Bañados & Fernandez, 1954
<i>B. elongata</i>	Nr
<i>B. fulva</i>	Nr
<i>B. hirsuta</i> Weinm.	Nr
<i>B. krugii</i>	Nr
<i>B. lingua</i>	Nr
<i>B. malabarica</i> Roxb.	- Bañados & Fernandez, 1954
<i>B. persichii</i>	Nr
<i>B. purpurea</i> L.	- Allen & Allen, 1981
<i>B. tomentosa</i> L.	- Allen & Allen, 1981
<i>Caesalpinia coriaria</i> (Jacq.) Willd.	- Bañados & Fernandez, 1954
<i>C. digyna</i>	Nr
<i>C. jayabo</i>	Nr
<i>C. nuga</i>	nr
<i>C. pulcherrima</i> (L.) Sw.	- Allen & Allen, 1981; - Moreira <i>et al.</i> , 1992
<i>C. sappan</i> L.	- Allen & Allen, 1936b
<i>C. tinctoria</i> HBK	nr
<i>Cassia alata</i> L.	- Bañados & Fernandez, 1954
<i>C. angustifolia</i> Vahl	nr
<i>C. divaricata</i> Nees & Bl.	nr
<i>C. fistula</i> L.	- Bañados & Fernandez, 1954
<i>C. hirsuta</i> L.	+ Keuchenius, 1924; - Steinmann, 1930
<i>C. grandis</i> L. f.	+ Wright cit. Allen & Allen, 1981; - Allen & Allen, 1936b; Moreira <i>et al.</i> , 1992
<i>C. javanica</i> L.	- Muller & Férmont cit. Allen & Allen, 1981
<i>C. laevigata</i> Willd = <i>C. floribunda</i> Cav.	- Steinmann cit. Allen & Allen, 1981
<i>C. leschenaultiana</i> DC.	+ Keuchenius, 1924
<i>C. mimosoides</i> L.	+ many investigators
<i>C. multijuga</i> Rich.	- Steinmann cit. Allen & Allen, 1981
<i>C. nodosa</i> Buch.-Ham. Ex Roxb.	- Allen & Allen, 1936b
<i>C. obtusifolia</i> L.	- many investigators
<i>C. occidentalis</i> L.	+ Keuchenius, 1924; - Muller & Férmont cit. Allen & Allen, 1981
<i>C. patellaria</i> DC.	+ Keuchenius, 1924
<i>C. pumila</i> Lam.	+ Muller & Férmont cit. Allen & Allen, 1981

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<i>C. siamea</i> Lam.	- Muller & Férmont cit. Allen & Allen, 1981
<i>C. sophora</i> L.	+ Grobbelaar & Clarke, 1967
<i>C. surattensis</i> Burm. f.	nr
<i>C. timorensis</i> DC.	- Steinmann cit. Allen & Allen, 1981
<i>C. tora</i> L.	- many investigators
<i>Copaifera langsdorffii</i> Desf.	nr
<i>Cynometra cauliflora</i> L.	- Allen & Allen, 1981
<i>C. chameecrista</i>	nr
<i>C. inaequalifolia</i>	nr
<i>C. ramiflora</i> L.	- Allen & Allen, 1981
<i>Delonix regia</i> (Boj. Ex Hook) Raf.	- Allen & Allen, 1936b
<i>Detarium senegalense</i> J. F. Gmel.	nr
<i>Dialium indum</i> L.	nr
<i>D. maingayi</i>	nr
<i>D. patens</i>	nr
<i>D. platysepalum</i>	nr
<i>Dipterix odorata</i> ( Aubl.) Willd.	- Allen & Allen, 1981
<i>Enderlia spectabilis</i> Steenis & de Wit	nr
<i>Gleditsia celebica</i> Koord.	nr
<i>Haematoxylon campechianum</i> L.	- Lim, 1977
<i>Humboldtia laurifolia</i> Vahl.	nr
<i>Intsia amboinensis</i>	nr
<i>I. bakeri</i> Prain	- Allen & Allen, 1981
<i>I. bijuga</i> (Colebr.) Ktze.	+ Norris, 1956; - Allen & Allen, 1981
<i>I. plurijuga</i> Harms	nr
<i>Kalappia celebica</i> Kosterm.	nr
<i>Koompassia malaccensis</i> Maing.	- Allen & Allen, 1981
<i>Manilkra scheffera</i> Schumm.	- Allen & Allen, 1981
<i>Mezoneurum pubescens</i>	nr
<i>Pahudia javanica</i> Miq.	nr
<i>Peltorum pterocarpum</i> Backer	- Allen & Allen, 1981
<i>P. dasyrhachis</i> Kurz.	nr
<i>P. grande</i>	nr
<i>Schizolobium exelsum</i> Vog.	- Bañados & Fernandez, 1954
<i>Sindora borneensis</i>	nr
<i>S. coriacea</i> Prain	nr
<i>S. javanica</i> (K. & V.) Backer	nr
<i>S. leiocarpa</i> Backer	nr
<i>S. parvifolia</i>	nr
<i>S. sumatrana</i> Miq.	nr
<i>S. velutina</i> Bak.	nr
<i>S. wallichii</i>	nr
<i>Tamarindus indica</i> L.	- Lim, 1977
<i>Tracylobium verrucosum</i> (Gaertn.) Oliv.	+ Wright cit. + Allen & Allen, 1981

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nr = no reports; + = nodulated; - = not nodulated.

Table 2. Nodulation in the subfamily Mimosoideae

Legume species	Nodulation and References
<i>Acacia arabica</i> Willd. = <i>A. nilotica</i> (L.) Willd. ex Del.	+ Allen & Allen, 1936a
<i>A. auriculiformis</i> A. Cunn. ex Benth.	+ Allen & Allen, 1936a
<i>A. baileyana</i> F. Muell.	+ many investigators
<i>A. borneensis</i> Nielsen	nr
<i>A. catechu</i> (L.f.) Willd.	+ Allen & Allen, 1936a
<i>A. concinna</i> (Willd.) DC.	nr
<i>A. cultriformis</i> A. Cunn. ex G. Don	+ Wilson. cit. Allen & Allen, 1981
<i>A. decurrens</i> Willd.	+ Allen & Allen, 1936a
<i>A. donnaiensis</i> Gagnep.	nr
<i>A. elata</i> A.Cunn. ex Benth.	+ Hannon cit. Allen & Allen, 1981
<i>A. falcata</i> Willd.	+ Norris, 1956
<i>A. farnesiana</i> (L.) Willd.	+ many investigators
<i>A. floribunda</i> (Vent.) Willd.	nr
<i>A. glauca</i> (L.) Moench.	nr
<i>A. holosericea</i> A. Cunn. ex G. Don	nr
<i>A. kekapur</i> Nielsen	nr
<i>A. kostermansii</i> Nielsen	nr
<i>A. leptocarpa</i> A. Cunn. ex Benth.	nr
<i>A. leucophloea</i> (Roxb.) Willd.	+ Allen & Allen, 1981
<i>A. linifolia</i> (Vent.) Willd.	+ Norris, 1956
<i>A. longifolia</i> (Andr.) Willd.	+ Harris cit. Allen & Allen, 1981
<i>A. longispicata</i> Benth. in Mitch.	nr
<i>A. mangium</i> Willd.	nr
<i>A. meansii</i> De Wild.	+ Corby, 1974
<i>A. megaladena</i> Desv.	nr
<i>A. merrillii</i> Nielsen	nr
<i>A. oraria</i> F. Muell.	nr
<i>A. pennata</i> (L.) Willd. = <i>A. megaladena</i> Merr.	+ Allen & Allen, 1981
<i>A. pluricapitata</i> Stendler ex Benth.	nr
<i>A. podalyriifolia</i> A. Cunn. ex G. Don	nr
<i>A. polybotrya</i> Benth.	nr
<i>A. pruinosa</i> A. Cunn. ex Benth.	nr
<i>A. pycnantha</i> Benth.	+ Harris cit. Allen & Allen, 1981
<i>A. rhetinoides</i> Schlecht.	+ Harris. cit. Allen & Allen, 1981
<i>A. stricta</i> (Andr.) Willd.	+ Lachmann cit. Allen & Allen, 1981
<i>A. suaveolens</i> (Sm.) Willd.	+ Norris, 1959b
<i>A. sulitii</i> Nielsen	nr
<i>A. tomentosa</i> Willd.	nr
<i>A. verheijanii</i> Nielsen	nr
<i>A. villosa</i> Willd. var. <i>glabra</i>	+ Keuchenius, 1924
<i>A. wetarensis</i> Pedley	nr
<i>Adenanthera borneensis</i> Brace ex Prain in King	nr
<i>A. forbesii</i> Gagnep.	nr
<i>A. kostermansii</i> Nielsen	nr
<i>A. malayana</i> Kosterm.	nr
<i>A. microsperma</i> Teijsm. & Binnend	nr
<i>A. pavonina</i> L.	- Allen & Allen, 1981; - Moreira <i>et al.</i> , 1992
<i>Albizia acle</i> (Blanco) Merr.	+ Allen & Allen, 1936a

<i>A. chinensis</i> (Osb.) Merr.	+ Allen & Allen, 1936a
<i>A. decurrens</i>	nr
<i>A. falcata</i> (L.) Backer	+ Wilson cit. Allen & Allen, 1981
<i>A. kostermansii</i> Nielsen	nr
<i>A. lobbeck</i> (L.) Benth.	+ many investigators
<i>A. lobbekoides</i> (DC.) Benth.	+ Allen & Allen, 1936a
<i>A. minahassae</i>	nr
<i>A. montana</i>	nr
<i>A. pedicellata</i> Baker & Benth.	nr
<i>A. procera</i>	+ Allen & Allen, 1936a
<i>A. retusa</i>	+ Allen & Allen, 1936a
<i>A. rotunda</i>	nr
<i>A. rufa</i> Benth.	nr
<i>A. saponaria</i> (Lour.) Blume ex Benth.	+ Allen & Allen, 1936a
<i>A. splendens</i> Miq.	nr
<i>Archidendron alatum</i> Pulle ex De Wit.	nr
<i>A. beguinii</i> De Wit.	nr
<i>A. borneense</i> (Benth.) Nielsen	nr
<i>A. minahassae</i>	nr
<i>A. montana</i>	nr
<i>A. pedicellata</i> Baker & Benth.	nr
<i>A. procera</i>	+ Allen & Allen, 1936a
<i>A. retusa</i>	+ Allen & Allen, 1936a
<i>A. rotunda</i>	nr
<i>A. rufa</i> Benth.	nr
<i>A. saponaria</i> (Lour.) Blume ex Benth.	+ Allen & Allen, 1936a
<i>A. splendens</i> Miq.	nr
<i>Archidendron alatum</i> Pulle ex De Wit.	nr
<i>A. beguinii</i> De Wit.	nr
<i>A. borneense</i> (Benth.) Nielsen	nr
<i>A. brachycarpum</i> Harms.	nr
<i>A. bubalinum</i> (Jacq.) Nielsen	nr
<i>A. clypcaria</i> (Jacq.) Nielsen	nr
<i>A. cockburnii</i> Nielsen	nr
<i>A. contortum</i> (Mart.) Nielsen	nr
<i>A. crateradenum</i> (Kosterm.) Nielsen	nr
<i>A. ellipticum</i> (Blume) Nielsen	nr
<i>A. fagifolium</i> (Blume ex Miq.) Nielsen	nr
<i>A. harmsli</i> V. Malm	nr
<i>A. havilandii</i> (Ridley) Nielsen	nr
<i>A. jiringa</i> (Jack) Nielsen	nr
<i>A. kalkmanii</i> (Kosterm.) Nielsen	nr
<i>A. kunstleri</i> (Prain) Nielsen	nr
<i>A. lucyi</i> F. Muell.	nr
<i>A. megaphyllum</i> Merr. & Perry	nr
<i>A. microcarpum</i> (Benth.) Nielsen	nr
<i>A. monopterum</i> (Kosterm.) Nielsen	nr
<i>A. nervosum</i> De Wit.	nr
<i>A. oppositum</i> (Miq.) Nielsen	nr
<i>A. palauense</i> (Kanch.) Nielsen	nr
<i>A. pauciflorum</i>	nr



<i>A. royerii</i> Kosterm.	nr
<i>A. sessile</i> (Scheffer) De Wit	nr
<i>A. tenuiracemosum</i> Kanch. & Hatus.	nr
<i>A. trichophyllum</i> (Kosterm.) Nielsen	nr
<i>A. triplinervium</i> (Kosterm.) Nielsen	nr
<i>Calliandra calothyrsus</i> Meissner	nr
<i>Cathomion umbellatum</i> (Vahl) Kosterm.	nr
<i>Dichros tachys cinerea</i> (L.) W. & A.	+ Corby, 1974
<i>Entada phaseoloides</i> (L.) Merr.	+ Wright cit. Allen & Allen, 1981
<i>E. rheedii</i> Spreng.	nr
<i>E. spiralis</i> Ridley	nr
<i>Entorobium saman</i> (Jacq.) Prain	nr
<i>Inga laurina</i> (Swartz) Willd.	+ Allen & Allen, 1936a
<i>L. leucocephala</i> (Lam.) De Wit = <i>L. glauca</i> (L.) Benth	+ Trinick, 1968
<i>Mimosa bimucronata</i> (DC.) O. Kuntze	+ Campêlo & Campêlo cit. + Allen & Allen, 1981
<i>M. diplotricha</i> C. Wright ex Sauvalle	nr
<i>M. invisita</i> Mart.	+ many investigators
<i>M. pigra</i> L.	+ DeSouza, 1966
<i>M. pudica</i> L.	+ many investigators
<i>Morobium monopterum</i> Kosterm.	nr
<i>Neptunia dimorphantha</i> Domin.	nr
<i>N. gracilis</i> Benth.	+ Bowen, 1956
<i>N. javanica</i> Miq.	nr
<i>N. oleracea</i> Lour.	+ Corby, 1974
<i>Pararchidendron pruinatum</i> (Benth.) Nielsen	nr
<i>Paraserianthes lophantha</i> (Willd.) Nielsen	nr
<i>P. falcata</i> (L.) Nielsen	nr
<i>Parkia biglobosa</i> (Jacq.) Benth.	nr
<i>P. intermedia</i> Hassk.	nr
<i>P. singularis</i> Miq.	nr
<i>P. speciosa</i> Hassk.	+ Lim, 1977
<i>P. sumatrana</i> Miq.	nr
<i>P. timoriana</i> (DC.) Merr.	nr
<i>P. versteeghii</i> Merr. & Perry	nr
<i>Pithecellobium affine</i>	nr
<i>P. angulata</i>	nr
<i>P. bubalinum</i>	nr
<i>P. clypearia</i>	nr
<i>P. dulce</i> (Roxb.) Benth.	+ many investigators
<i>P. ellipticum</i> (Bl.) Hassk.	+ Allen & Allen, 1981
<i>P. fagifolium</i>	nr
<i>P. kunstleri</i>	nr
<i>P. lobatum</i>	nr
<i>P. minahassae</i>	nr
<i>P. umbellatum</i>	nr
<i>Serianthes grandiflora</i> (Wall.) Benth.	nr
<i>S. hooglandii</i> (Fosb.) Kanis	nr
<i>S. minahassae</i> (Koord.) Merr. & Perry	nr
<i>S. robinsonii</i>	nr
<i>Wallaceodendron celebicum</i> Koord.	+ Allen & Allen, 1981

nr = no reports; + = nodulated; - = not nodulated.

Table 3. Nodulation in the subfamily Papilionoideae

Legume species	Nodulation and References
<i>Abrus laevigatus</i> E. Mey	+ Keuchenius, 1924
<i>A. modesta</i>	nr
<i>A. oraria</i>	nr
<i>A. precatorius</i> L.	+ many investigators
<i>Aeschynomene americana</i> L. var. <i>javanica</i>	+ Keuchenius, 1924
<i>A. falcata</i> DC.	+ Keuchenius, 1924
<i>A. indica</i> L.	+ many investigators
<i>Alysicarpus ludens</i> Wall.	+ Keuchenius, 1924
<i>A. nummulariaefolius</i> DC.	+ Keuchenius, 1924
<i>Arachis hypogaea</i> L.	+ Staphorst & Strijdom, 1972
<i>Atylosia scarabaeoides</i> (L.) Benth.	+ Chen & Shu, 1944
<i>A. barbata</i> W. & A.	+ Lechtova-Trnka cit. Allen & Allen, 1981
<i>Butea monosperma</i> (Lam.) Taub.	- Allen & Allen, 1981
<i>Cajanus cajan</i> (L.) Millsp.	+ Allen & Allen, 1939
<i>Calopogonium mucunoides</i> Desv.	+ Lim, 1977
<i>Canavalia ensiformis</i> (L.) DC.	+ Saono <i>et al.</i> , 1975
<i>C. gladiata</i> (Jacq.) DC.	+ Saono <i>et al.</i> , 1975
<i>C. rosea</i>	nr
<i>Centrolobium</i> sp.	nr
<i>Centrosema plumieri</i> (Turp.) Benth.	+ Bañados & Fernandez, 1954
<i>C. pubescens</i> Benth.	+ many investigators
<i>C. quinquefolia</i>	nr
<i>C. virginianum</i> (L.) Benth.	+ Keuchenius, 1924
<i>Clitoria laurifolia</i> Poir.	+ Dubey <i>et al.</i> , 1972
<i>C. tematea</i> L.	+ many investigators
<i>Crotalaria acicularis</i> Buch.-Ham. ex Benth.	+ Keuchenius, 1924
<i>C. alata</i> Buch.-Ham.	+ many investigators
<i>C. anagyroides</i> HBK	+ many investigators
<i>C. ferruginea</i>	nr
<i>C. juncea</i> L.	+ many investigators
<i>C. pseudo-eriosema</i> Vatke	+ Keuchenius, 1924
<i>C. retusa</i> L.	+ many investigators
<i>C. striata</i> DC.	+ many investigators
<i>C. usaromoensis</i> Bak. f.	+ many investigators
<i>C. vaeletonii</i> Backer	+ many investigators
<i>Cytisus palmensis</i> (Christ.) Hutch.	nr
<i>Dalbergia latifolia</i> (Roxb.)	+ Allen & Allen, 1981
<i>D. parviflora</i>	nr
<i>D. pinnata</i>	nr
<i>D. sissoo</i> Roxb.	+ Allen & Allen, 1981
<i>Derris elliptica</i> (Roxb.) Benth.	+ many investigators
<i>D. heptaphylla</i> Merr.	nr
<i>D. heterophylla</i>	nr
<i>D. junghuhnii</i>	nr
<i>D. microphylla</i> (Miq.) Val.	+ Allen & Allen, 1936a
<i>D. polyphylla</i> Koord. & Val.	nr
<i>Desmodium auriculatum</i> DC. = <i>Pteroloma auriculatum</i> (DC.) Schindl.	+ Keuchenius, 1924

<i>D. capitatum</i> DC.	+ Keuchenius, 1924
<i>D. elegans</i> Benth.	+ Keuchenius, 1924
<i>D. gangeticum</i> (L.) DC.	+ many investigators
<i>D. gyroides</i> DC. = <i>Codariocalyx gyroides</i> (Roxb.) Hassk.	+ many investigators
<i>D. heterocarpon</i> (L.) DC.	+ Keuchenius, 1924
<i>D. heterophyllum</i> (Willd.) DC.	+ many investigators
<i>D. intortum</i> Urb.	+ Allen & Allen, 1981
<i>D. latifolium</i> DC.	+ Keuchenius, 1924
<i>D. laxiflorum</i> DC.	+ Bañados & Fernandez, 1954
<i>D. pulchellum</i> (L.) Benth = <i>Phyllodium pulchellum</i> (L.) Desv.	+ Keuchenius, 1924
<i>D. purpureum</i> H. & A.	+ many investigators
<i>D. sequax</i>	nr
<i>D. timorensis</i>	nr
<i>D. triflorum</i> (L.) DC.	+ many investigators
<i>D. triquetrum</i> (L.) DC = <i>Pteroloma triquetrum</i> (DC.) Benth.	+ Keuchenius, 1924
<i>D. umbellatum</i> (L.) DC	+ Allen & Allen, 1981
<i>Dipteryx odorata</i> (Aubl.) Willd.	- Allen & Allen, 1981
<i>Dolichos biflorus</i> L.	+ many investigators
<i>D. lablab</i> L. = <i>Lablab purpureus</i> (L.) Sweet	+ Wilson, 1939a
<i>Erythrina euodiphylla</i>	nr
<i>E. fusca</i> Lour.	+ Allen & Allen, 1936
<i>E. lithosperma</i> Miq. = <i>E. subumbrans</i> (Hassk.) Merr.	+ Allen & Allen, 1936a
<i>E. microcarpa</i>	nr
<i>E. micropterix</i> Poepp.	+ DeSouza, 1966
<i>E. variegata</i> L.	+ Bañados & Fernandez, 1954
<i>Euchresta horsfieldii</i> (Lesch.) Benn.	nr
<i>Flemingia congesta</i> Roxb.	+ Allen & Allen, 1973
<i>F. lineata</i> Roxb.	+ Keuchenius, 1924
<i>F. strobilifera</i> (L.) Ait.	+ Bañados & Fernandez, 1954
<i>Galactia tenuiflora</i> (Willd.) W. & A.	+ Keuchenius, 1924
<i>Gliricidia sepium</i> (Jacq.) Steud.	+ Holland cit., Allen & Allen, 1981
<i>Glycine max</i> (L.) Merr.	+ many investigators
<i>Glycyrrhiza glabra</i> L.	+ Morck cit. Allen & Allen, 1981
<i>Hanslia adhaerens</i> (Poir.) Schindl.	nr
<i>Indigofera arrecta</i> Hochst. ex A. Rich	+ many investigators
<i>I. ende caphylla</i> Jack.	+ many investigators
<i>I. galegoides</i>	nr
<i>I. hendecaphylla</i>	nr
<i>I. hirsuta</i> L.	+ many investigators
<i>I. suffruticosa</i> Mill.	+ many investigators
<i>I. sumatrana</i> Gaertn.	+ Wilson, 1939a
<i>Inocarpus edulis</i> Forst.	- Allen & Allen, 1981
<i>Kunstleria curtisii</i> Prain	nr
<i>Lupinus spectabilis</i> Hoover	+ Allen & Allen, 1981
<i>Macroptilium atropurpureum</i> Urb.	+ Allen & Allen, 1939
<i>Mastersia bakeri</i> (Koord.) Bak.	+ Keuchenius, 1924
<i>Mecopus nidulans</i> Benn.	nr
<i>Medicago sativa</i> L.	+ many investigators
<i>Milletia atropurpurea</i> Benth.	nr
<i>M. dasyphylla</i>	nr
<i>M. sericea</i> Benth.	nr

<i>Mucuna cyanosperma</i>	nr
<i>M. diabolica</i> Backer & Heyne	+ Keuchenius, 1924
<i>M. junghuhniana</i>	nr
<i>M. pruriens</i> (L.) DC.	+ Keuchenius, 1924
<i>Myroxylon balsamum</i> (L.) Harms	+ Allen & Allen, 1981
<i>Ormocarpum sennoides</i>	nr
<i>Ormosia bancana</i>	nr
<i>O. calavensis</i>	nr
<i>O. macrodisca</i>	nr
<i>O. sumatrana</i> (Miq.) Prain	+ Büsgen cit. Allen & Allen, 1981
<i>Pachyrhizus erosus</i> (L.) Urb.	+ Bañados & Fernandez, 1954
<i>Padbruggea dasyphylla</i> Mq.	nr
<i>Parochetus communis</i> Buch.-Ham. ex D. Don	+ Grobbelaar & Clarke, 1974
<i>Pericopsis</i> sp.	nr
<i>Phaseolus atropurpureus</i> Moc. & Sessé	+ Saono <i>et al</i> , 1975
<i>Ph. calcaratus</i> Roxb. = <i>Vigna umbellata</i> (Thunb.) Ohwi & Ohashi	+ many investigators
<i>Ph. lunatus</i> L.	+ many investigators
<i>Ph. mungo</i> L. = <i>Vigna mungo</i> L.	+ many investigators
<i>Ph. radiatus</i> L. = <i>Vigna radiata</i> (L.) Willczek	+ many investigators
<i>Ph. scaberulus</i>	nr
<i>Ph. semi-erectus</i> L.	+ Wilson, 1945
<i>Ph. sublobatus</i> Roxb.	+ Keuchenius, 1924
<i>Ph. vulgaris</i> L.	+ many investigators
<i>Phyllodium pulchellum</i> (L.) Desv.	+ Keuchenius, 1924
<i>Pisum arvense</i> L.	+ many investigators
<i>P. sativum</i> L.	+ many investigators
<i>Pongamia pinnata</i> (L.) Pierre	+ Wright cit. Allen & Allen, 1981
<i>Pseudarthria viscida</i> (L.) W. & A.	+ Keuchenius, 1924
<i>Psophocarpus palustris</i> Desv.	+ Keuchenius, 1924
<i>P. tetragonolobus</i> (L.) DC.	+ Keuchenius, 1924
<i>Pterocarpus indicus</i> Willd.	+ Bañados & Fernandez, 1954
<i>P. santalinus</i> L. f.	nr
<i>Pteroloma auriculatum</i> (DC.) Schindl.	+ Keuchenius, 1924
<i>P. triquetrum</i> (DC.) Benth.	+ Keuchenius, 1924
<i>Pueraria phaseoloides</i> (Roxb.) Benth.	+ Keuchenius, 1924
<i>P. triloba</i> (Lour.) Makino	+ Keuchenius, 1924
<i>Rhynchosia mollissima</i> (Eli.) Schuttler ex W. Wats	+ Keuchenius, 1924
<i>R. phaseoloides</i> DC.	+ Keuchenius, 1924
<i>R. rufescens</i> (Willd.) DC.	+ Keuchenius, 1924
<i>Sesbania grandiflora</i> Poir.	+ many investigators
<i>S. rostrata</i> Brem. & Oberm.	+ Corby, 1974
<i>S. sesban</i> (L.) Merr.	+ Corby, 1974
<i>Shuteria vestita</i> W. & A.	+ Keuchenius, 1924
<i>Smithia sensitive</i> Ait.	+ Allen & Allen, 1981
<i>Sophora japonica</i> L.	+ Allen & Allen, 1981
<i>S. tomentosa</i> L.	+ Allen & Allen, 1981
<i>Spatholobus ferrugineus</i>	nr
<i>S. littoralis</i> Hassk.	nr
<i>Tephrosia candida</i> (Roxb.) DC.	+ many investigators
<i>T. maxima</i> Pers.	+ Keuchenius, 1924
<i>T. noctiflora</i> Boj. ex Bak.	+ many investigators

<i>T. purpurea</i>	+ many investigators
<i>T. villosa</i> (L.) Pers.	+ Allen & Allen, 1981
<i>T. vogeli</i> Hook. f.	+ many investigators
<i>Teramnus labialis</i> (L. f.) Spreng.	+ Keuchenius, 1924
<i>Trifolium alexandrinum</i> L.	+ many investigators
<i>T. repens</i> L.	+ many investigators
<i>Trigonella foenum-graecum</i> L.	+ many investigators
<i>Uraria crinata</i> Desv.	nr
<i>U. lagopodioides</i> (L.) Desv. Ex DC.	+ Bañados & Fernandez, 1954
<i>U. spinosa</i> Desv.	+ Allen & Allen, 1981
<i>Vicia faba</i> L.	+ many investigators
<i>Vigna cylindrical</i> (L.) Skeels	+ Saono <i>et al.</i> , 1975
<i>V. hosei</i> (Craib) Backer	+ Allen & Allen, 1981
<i>V. marina</i> (Burm.) Merr.	+ Keuchenius, 1924
<i>V. sinensis</i> (L.) Endl. Ex Hassk.	+ many investigators
<i>V. vexillata</i> (L.) A. Rich	+ McLeod, 1962
<i>V. vilosa</i>	+ Keuchenius, 1924
<i>Voandzeia subterranean</i> Thou.	+ DeSouza, 1963

nr = no reports; + = nodulated; - = not nodulated.

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**BIODIVERSITY OF *PSEUDOMONAS* SPP.  
ISOLATED FROM PLANT RHIZOSPHERES AND THEIR POTENTIAL USE  
AS PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)**

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**Introduction**

In natural ecosystem equilibrium develops between the plant and microorganisms that is affected by the growth of plants and seasonal changes in the environment. The association between organism and roots can be beneficial, harmful or neutral, but often the effects depend on soil conditions and must therefore be regarded as variable. Recently, many attempts were made to manipulate the rhizosphere to increase the balance over the harmful effects. The common approach to the control of the soil and the rhizosphere are the introduction of specific antagonistic microorganisms into the soil, on seed surface or around tubers. These antagonistic bacteria found on plant roots, are termed as plant growth promoting rhizobacteria (PGPR). Most PGPR are members of fluorescent pseudomonads, but other types have also been found. Growth promotion is evidenced by increase in seedling emergence, plant vigor, seedling weight, root system development, and yield. The production by PGPR of antibiotics and siderophore, which are active against deleterious microorganisms, has been proposed as a mechanism for the plant growth promotion. Therefore, the use of certain species of *Pseudomonas* spp. to improve plant growth is quite promising and thus has to be explored and evaluated carefully.

In this experiment, the root microorganisms and particularly the bacteria were isolated from the rhizospheres of graminaceous/cereals, grass, and legumes. Because the most common reported rhizobacteria are *Pseudomonas* spp., the media used for isolation are the selective media for *Pseudomonas* spp. The *Pseudomonas* selective medium used is MKB (Modified King B) medium, which has been used regularly by many researchers (Anas *et al.*, 1987a and 1987b; Arif, 1990 and 1995; Hofte, *et al.*, 1991). To obtain a good inoculum, special attention has to be given to the isolates during isolation, effectiveness test or screening test, and preservation of the good or prospective selected strains.

**Objectives and Benefit of The Research**

*Objectives*

The main objectives of the experiment were (1) to isolate rhizobacterial strains of *Pseudomonas* spp. from rhizospheres of graminaceous/cereals, grass, and legumes, (2) to select the rhizobacterial strains of *Pseudomonas* spp. for their capacity to increase plant growth, (3) to verify the selected rhizobacterial strains of *Pseudomonas* spp. for their plant growth promoting capacity, and (4) to identify the selected beneficial rhizobacterial strains of *Pseudomonas* spp.

*Benefit of research*

Production an effective inoculum by using *Pseudomonas* spp. as novel microorganisms might support a high agricultural production or even could be used as biocontrol agents for some soil-borne diseases in various types of plants (food crops, horticultural plants, and forest seedlings or trees). The use of plant growth promoting rhizobacteria (PGPR) in agricultural production system may significantly balance intensive applications of chemical fertilizers and pesticides.

Seed inoculation with beneficial *Pseudomonas* spp. apparently escapes from attention of many researchers. Improvement of inoculation technique and inoculum formulation is expected to attract farmers to use *Pseudomonas* spp. in their agricultural practices. For example, result from the

research could be further investigated in formulating suitable carrier(s) for the inoculants such as peat materials, powdered coconut husk, dried compost, mineral soils, clay materials (kaolinite, bentonite, etc.). Incorporating agricultural waste materials as suitable carrier (s) for the inoculum production might solve the environmental problems.

### Literature Review

Rhizosphere is composed of three zones. The rhizosphere in a strict sense consists of the soil around the roots in which soluble and volatile compounds excreted by the roots diffuse. The rhizoplane comprises the root surface and mucigel covering part of the roots behind the root cap. The endorhizosphere consists of epidermis and cortex cells invaded by microorganisms.

The term of "rhizobacteria" was first proposed by Kloepper and Schroth in 1978 to point out bacteria that live in a close relation with plant roots. The terms rhizoplane or rhizosphere bacteria are used to describe bacterial groups isolated from rhizoplane or rhizosphere, irrespective of their function or their effective population density at that sites. Therefore, it is possible that the so-called rhizosphere or rhizoplane bacteria are not really root colonizers but they have been transiently present and isolated by chance.

Schroth and Hancock (1981) divided rhizobacteria into three value groups with regard to plant growth, namely, beneficial, neutral, and deleterious. The potential of beneficial rhizobacteria as seed inoculants has attracted soil microbiologists and plant pathologists, especially, because of their aggressive behavior in colonizing plant roots and displacing pathogenic, quasi-pathogenic or deleterious rhizosphere microorganisms.

Fluorescent pseudomonads are important components of the rhizosphere of many plants. They may function as antagonists of some pathogens in the soil and the rhizosphere of plants. Several mechanisms by which rhizobacterial strains of *Pseudomonas* spp. enhance the plant growth have been reported (Burr and Caesar, 1984). The mechanisms may include (a) the inoculated strains preemptively colonize the rhizosphere of plants and thus exclude the populations of deleterious microorganisms; (b) the inoculated strains produce plant growth promoting substances; (c) the inoculated strains mineralize soil phosphate, and (d) the inoculated strains antagonize pathogens, quasi-pathogens, or other deleterious microorganisms by production of extra cellular microbial metabolites (Table 1).

Besides some evidence on the potential use of *Pseudomonas* spp. some failures of their use may be fruitful to be highlighted here. Fluorescent pseudomonads isolated from the rhizosphere of sugar beet have been shown to reduce significantly the growth of sugar beet and wheat (Elliot and Lynch, 1984). In the case of cereals (wheat) and probably also other crops, *Pseudomonas* spp. colonized plant roots and even produced toxins to retard other rhizosphere microorganisms and also plant growth. In addition, fluorescent pseudomonads inhibited the growth of horticultural plants such as rough lemon and sweet orange seedling (Gardner et al., 1984).



Table 1. Various mechanisms by which *Pseudomonas* spp. promote the plant growth (after Anas, 1986)

Mechanisms	Species	References
a. Niche exclusion:		
1. Potato	<i>P. fluorescens</i>	Burr <i>et al.</i> (1978)
	<i>P. putida</i>	Burr <i>et al.</i> (1978)
2. Sugar beet	<i>Pseudomonas</i> spp	Kloepper <i>et al.</i> (1978)
b. Production of plant promoting substances:		
1. Auxines	<i>Pseudomonas</i> spp	Lutenberg <i>et al.</i> (1971)
	<i>P. fluorescens</i>	Stone and Strominger (1971)
2. Gibberellins	<i>Pseudomonas</i> spp.	
	<i>P. fluorescens</i>	
3. Gibberellins-like substances	<i>Pseudomonas</i> spp.	Eklund (1970)
	<i>P. fluorescens</i>	Katznelson and Cole (1965)
4. Vitamins	<i>P. fluorescens</i>	Hussain and Vancura (1970)
c. Production of phosphate-solubilizing compounds:		
	<i>P. striata</i>	
	<i>P. putida</i>	Grimes and Mount (1984)
d. Antagonisms:		
1. Siderophores	<i>P. fluorescens</i>	Scher and Baker (1980)
	<i>P. putida</i>	Scher and Baker (1980)
		Teintze <i>et al.</i> (1981)
	<i>P. fluorescens</i>	Kloepper <i>et al.</i> (1980 a,b)
		Arif (1990; 1995)
	<i>P. aeruginosa</i>	Anas (1987 a,b)
		Arif (1990; 1995)
		Hofte <i>et al.</i> (1991)
		Devliegher <i>et al.</i> (1996)
2. Antibiotics	<i>P. fluorescens</i>	Howel and Stipanovic (1979)
	<i>Pseudomonas</i> spp.	Digat (1983)

## Materials and Methods

### Collection of the rhizosphere samples

The rhizospheres of graminaceous/cereals, grass, and legumes were sampled at 35 sites from 37 locations in Yogyakarta regions. The root of growing cane (*Saccharum officinarum*), maize (*Zea mays*), rice (*Oryza sativa*), Gajah grass (*Pennisetum purpureum*), Kolonjono grass (*Brachiaria mutica*), bean (*Phaseolus vulgaris*), long bean (*Pisum sativum*), soybean (*Glycine max*), and peanut (*Arachis hypogaea*) were collected directly from the fields with a spade or hoe. The root samples were stored in a thick plastic bag, tied loosely, and punctured to facilitate enough aeration. Each samples was coded according to the origins of plant and location.

To ensure the freshness of the root samples during transportation to the laboratory, the root samples were kept in an ice bucket or polyethylene thermos, and then transferred immediately into a refrigerator at 2 – 4° C. When root samples were ready for isolation, the samples were taken from refrigerator and isolation was immediately carried out.

#### *Isolation and purification of the rhizobacterial strains of Pseudomonas spp.*

The roots of various plants, respectively, were washed gently with tap water and then cut into pieces of ca 0.5 cm with a pair of scissors. Then, the roots were mixed with sterile quartz-sands and ground with a porcelain mortar. A ten-fold dilution series was subsequently prepared in saline solution (8.5 g NaCl in 1 liter sterile distilled water). The appropriate dilution series were plated out.

The Modified King B (MKB) medium was used for routine isolation (Anas, 1986). The plates were incubated at temperature of 28 °C. The criteria used for selection of rhizobacterial isolates are as follows.

1. The production of dissolved pigment on MKB medium, such as, green, blue, brown, yellow, or even colorless;
2. The colony size which reflects, one way or another, a growth rate of the isolate; and
3. The clear inhibition zones reflecting antagonistic capacity of the rhizobacterial strain towards other microorganisms.

According to the afore-mentioned criteria, at least three isolated colonies from each sample were selected and then further purified on MKB medium. After the incubation for 3 days at 28 °C, the isolates were kept in a refrigerator to slowdown their growth prior to conducting effectiveness tests. Regular transfer of each isolates was carried out. To avoid contamination, the purification of isolates was done in replicates, especially, for the good or prospective isolates.

#### *Effectiveness test of the isolated rhizobacterial strains (greenhouse experiment)*

Series of greenhouse experiment were carried out to evaluate the effects of inoculation. The average dry weight of plants originated from the inoculated seeds was compared to the uninoculated seeds (control treatment). Each pair of treatment was then analyzed statistically using Student's t-test. There were three possible effects of the plant test: (a) the inoculation increased dry weight of plants at a significant level of 5%; (b) the inoculation had no significant effect; or (c) the inoculation significantly decreased the dry weight of plants.

In order to perform the plant experiments, some activities were carried out as follows.

#### Preparation of the inoculum

Each rhizobacterial isolate was cultured in a liquid MKB medium and shaken (ca 120 rpm) on a rotary shaker for 3 days at 28 °C. After incubation, the cells were harvested by centrifugation (5,000 x g) for 30 minutes. The cell pellets were washed thoroughly with saline solution and resuspended in 1% (w/v) methyl cellulose (CMC) solution. This solution containing rhizobacterial cells ( $10^7$ -  $10^8$  cfu/ml) was used to inoculate seeds.

#### Seed inoculation procedure

Certified maize cultivar Bisi-2 was used as a test plant in this experiment. The maize seeds were surface sterilized by immersion in 70% (v/v) ethanol for 10 seconds followed by a soak in 5% (v/v) hydrogen peroxide ( $H_2O_2$ ) for 2 minutes, and then rinsed 3 times in sterile distilled water. The surface sterilized-seeds were submerged in viable rhizobacterial cells for the inoculated treatments and in autoclaved cells for the control treatments. The inoculated and control seeds, respectively, were sown into the soil.

#### Greenhouse experiment

Soil taken from Patuk, Gunung Kidul, was used as a medium during the effectiveness test. The soil was air dried and passed through a 2 mm-sieve. One kg of completely mixed soil was filled into a 1 liter-plastic pot. Twenty ml of 5 time-concentrated Long Ashton nutrient solutions were added to each pot (Hewitt, 1996).

Both the inoculated and noninoculated (control) maize seeds were sown into the prepared soil at ca. 2 cm depth. The soil water content was adjusted to  $\frac{3}{4}$  water holding capacity (30% dry basis) by

adding water to the pot soil, once a day. Each treatment was repeated 4 times. The plants were grown for 3 weeks in the greenhouse.

The effect of inoculations was measured by comparing the dry weight of the inoculated plants to the average dry weight of the corresponding control. To determine the plant dry weight, plant materials were kept in a paper bag and dried in the oven at 70 °C for 48 hours. The students t-test was used for statistical analyzes. The rhizobacterial strains that significantly increased plant dry weight were selected for further experiments.

#### Preservation of the effective rhizobacterial strains

The effective rhizobacterial strains resulted from the greenhouse experiments were preserved on the MKB slant and kept in a refrigerator at 2-4 °C. Regular transfers of the strains were conducted for at least every 2 or 3 weeks.

#### Verification study of the selected rhizobacterial strains of *Pseudomonas* spp. for their plant growth promoting capacity

The objectives of this experiment were to evaluate results obtained from the effectiveness experiment of selected strains of *Pseudomonas* spp. and to select several from amongst best strains further investigation in the future.

The procedures to conduct this experiment were essentially the same as the effectiveness test experiment. For example, preparation of the selected strains, seed inoculation procedure, and greenhouse experiment had been explained clearly in the previous section.

#### Identification of the selected rhizobacterial strains of *Pseudomonas* spp.

The standard procedure of Bergey's Manual was used to identify the selected rhizobacterial strains of *Pseudomonas* spp. Qualitative determination of bacterial species included observation of cell morphology and motility, flagellar arrangement, nature of produced pigment (siderophores), growth on different temperatures, the ability to assimilate some carbon sources, organic acids, amino acid or amines, and SDS-page of selected beneficial strains of *Pseudomonas* spp.

#### Plate assay for the growth of the PGPR on carbon sources, organic acids, amino acids or amines

To determine the ability of PGPR isolates to grow on 12 carbohydrates, 4 organic acids, and 18 amino acids or amines, solid M9 mineral medium was used (Maniatis *et al.*, 1982). Each carbohydrates and organic acids was supplied to M9 mineral medium as a sole carbon source and was given at a concentration of 0.8 – 2.0 g C l<sup>-1</sup>

Amino acids or amines were supplied as both carbon and nitrogen sources at a concentration of 0.8 – 2.0 g C l<sup>-1</sup> and of 0.26 – 0.58 g N l<sup>-1</sup>. Bacterial colony previously grown on soil MKB medium was streaked on solid M9 mineral medium containing each carbon and/or nitrogen resources. Plates were incubated at 28° C for 3 days. Respectively, the growth of PGPR on 12 carbohydrates, 4 organic acids, and 18 amino acids or amines were observed on plates under the UV lamp. The visual colonies on the plates were recorded as a positive growth. *Pseudomonas aeruginosa* 7 NSK2 strain, a plant growth promoting rhizobacteria isolated by Anas (1987 a; b), was used as a reference isolate.

#### Effect of incubation temperature on the growth and siderophore production by PGPR isolates

Bacterial cell cultures previously grown in MKB broth at a late exponential phase were strike on MKB and MKB + EDTA (1.0 g l<sup>-1</sup>) solid medium. Plates were incubated at ambient temperatures at 4°C, 12.5°C, 28°C, 33 °C, and 42 °C.

The siderophore production on plates at different temperatures was checked under the UV lamp. The visual colonies on plates were recorded as a positive growth. *Pseudomonas aeruginosa* strain 7 NSK2 and *Pseudomonas fluorescens* ANP15 (Anas, 1987 a; b) were used as a reference isolate.

#### Qualitative determination of siderophores produced by the PGPR (Scher and Baker, 1982)

To determine siderophore production, M9 mineral medium containing 2 g glucose-C1<sup>-1</sup> was used. Iron was added (0.1 g FeCl<sub>3</sub> per liter M9 medium) in the half of treatments. The test organisms were introduced into 50-ml portion of the medium and incubated on a rotary shaker (175 rpm) at the temperature of 28° C. After 24 hours, the cultures were centrifuged at 4.000 x g for 30 minutes. Supernatant was filtered through a 0.2 um filter to remove the residual cells and the pH of the filtrate was adjusted to 5.5.

Each supernatant was added to 2 spectrophotometer tubes (3 ml per tube) fifteen µl of 0.01 M Fe Cl<sub>3</sub> was added to one tube, whereas the other tube, to which no iron was added, served as a blank. Absorbance at various wavelengths was read against the blank.

#### Bacterial cell membrane preparations and SDS-Page

Outer membranes of late-exponential phase cells were obtained using a modified method of Cornelis (Seong, 1991). Three ml of cells grown in different media (LB: Luria Broth, M9 + citrate, and M9 + glucose) were harvested by centrifugation at 14,000 x g for 2 minutes. The pellets were incubated in the presence of 100 µl lysozyme and EDTA (8 mg lysozyme in 2 ml EDTA lysis buffer) for 30 minutes. To lyse the cells completely, 100 µl 2 % N-Lauroyl Sarcosine (NLS, dissolved in 25 mM Tris-HCl buffer, pH 8.0) was added followed by the addition of 300 µl 1% NLS. The cell suspensions were sheared with syringes until the viscosity of the suspension disappeared, and were centrifuged twice at 14,000 x g for 30 seconds to remove cell debris. The supernatant containing the membrane fraction was centrifuged at 14,000 x g for 30 minutes. This pellet was washed with 250 µl NLS (1%) and suspended in 30 µl Tris-HCl buffer (10 mM, pH 7.8). Thirty µl loading buffer containing 0.63 M Tris-HCl (pH 6.8), 10% glycerol (v v<sup>-1</sup>), 2% sodium dodecyl sulfate (SDS, w v<sup>-1</sup>), 5% β-mercaptoethanol (v v<sup>-1</sup>), and 0.0025% bromophenol blue was added.

SDS polyacrilamide gel electrophoresis was carried out as described by Harlow and line (1988) using a Mini-PROTEAN II dual slab cell (Bio-Rad). Separating gels contained 15% (w v<sup>-1</sup>) acrylamide mix (14.6 % acrylamide and 0.4 % N,N'-methylene-bis-acrylamide), 0.1% SDS, 0.04% TEMED (v v<sup>-1</sup>), N,N,N',N'-Tetramethylethylenediamine in 0.125 M Tris-HCl (pH 6.8). Electrophoresis was carried out at 50 mA per slab 7 cm x 8 cm x 0.75 mm).

## Result and Discussion

### *Collection of the rhizosphere and soil samples*

Locations, number of rhizosphere samples and plant species used for isolation of rhizopseudomonads were presented in Table 2. Rhizospheres of various plants were collected from 35 sites distributed throughout 7 locations in Yogyakarta regions.

Some important physicochemical properties of the rhizosphere soils are presented Table 3. Result of rhizosphere soil analysis indicated that each location represented different soil and/or ecophysiological conditions.

### *Isolation and purifications of Pseudomonas spp.*

Since the root samples were kept in an icebox during transportation from the fields, the roots were still fresh then they arrived at the laboratory. This was very important to avoid the death of rhizosphere microorganism. At least 3 isolates from each rhizosphere samples were selected. Two or three days after the incubation at 28 – 30 °C on solid MKB medium, the rhizobacterial colonies appeared. Based on the criteria (size of colony, inhibition zone, and production of pigment), the isolated colonies were selected. Therefore, 136 strains from 35 sites from 7 locations were isolated from the rhizosphere of graminaceous/cereals, grass, and legumes (data not shown). Seventy-eight isolates (57.4%) produced fluorescent pigment or siderophores, 49 isolates (36.0 %) were not pigmented (colorless or hyaline) and 9 isolates (6.4%) produces non-fluorescent yellow pigment. Most of the isolates (97 strains or 71%) exhibited medium to strong clear inhibition zone (data not shown).

*Effectiveness test of the rhizopseudomonad isolates (greenhouse experiment)*

Soil used for medium to grow maize plants in the greenhouse was taken from Patuk, Gunung Kidul, Yogyakarta. Chemical composition and physicochemical properties of Patuk soil are shown in Table 4. In general, soil had a medium pH, heavy texture, and a very low P content.

Table 2. Locations, number of rhizosphere samples, and plant species/origin for the isolation of rhizopseudomonads

Locations	Number of samples	Plant Species
Bantul	4	Soybean ( <i>Glycine max</i> ) Kolojono grass ( <i>Rachiaria mutia</i> ) Maize ( <i>Zea mays</i> ) Peanut ( <i>Arachis hypogea</i> )
Gamping	5	Soybean ( <i>Glycine max</i> ) Kolojono grass ( <i>Rachiaria mutia</i> ) Maize ( <i>Zea mays</i> ) Bean ( <i>Phaseolus vulgaris</i> ) Cane ( <i>Saccharum officinarum</i> )
Sedayu	5	Long bean ( <i>Pisum sativum</i> ) Maize ( <i>Zea mays</i> ) Paddy rice ( <i>Oryza sativa</i> )
Sentolo	7	Sorghum ( <i>Sorghum bicolor</i> ) Kolojono grass ( <i>Rachiaria mutia</i> ) Maize ( <i>Zea mays</i> )
Sewon	1	Kolojono grass ( <i>Rachiaria mutia</i> )
Ternon	7	Long bean ( <i>Pisum sativum</i> ) Peanut ( <i>Arachis hypogea</i> ) Cane ( <i>Saccharum officinarum</i> )
Wates	6	Gajah grass ( <i>Pennisetum purpureum</i> ) Kolonjono grass ( <i>Brachiaria mutica</i> ) Maize ( <i>Zea mays</i> )
Total	35 samples	9 plant species

Forty-eight isolates had been tested for their ability to enhance growth of maize cultivar Bisi-2 in the greenhouse. Five rhizopseudomonad isolates (44, 66, 67, 71, and 126). Increased plant dry weight from 19% up to 25%, 4 isolates decreased plant dry weight from 14% up to 27%, and the other isolates were neutral (Table 5). All of these PGPR isolates produced siderophores and were originated from maize rhizosphere. However, all deleterious isolates were originated from rhizosphere of cane, grass, and peanut. This observation clearly demonstrates the presence of plant-microbe specificity phenomenon.

*Growth of PGPR on carbohydrates, organic acids, and amino acids or amines*

To study physiological characteristics of the PGPR isolates, carbon and nitrogen assimilation tests were conducted. Each PGPR strains was tested for their ability to grow on 12 carbohydrates, 4 organic acids, and 18 amino acids or amines (data not shown). Results presented in Table 6 clearly show that 5 PGPR isolates are grouped into 4 different strains (44, 66, 67, & 71, and 126). All PGPR isolates were unable to grow on betaine, glycine, and DL-threonine, suggesting that all PGPR isolates are most likely not members of *Pseudomonas aeruginosa*. Our finding is very important to ensure safety use of these PGPR isolates in the future. Yet, *P. aeruginosa* is commonly found in soil and causes root rot of some plants (Bergey's, 1984). Clinical isolates of these bacteria are also known to be opportunistic human pathogens.

Table 3. Some important physicochemical properties of the rhizosphere soils

Soil Properties	Location					
	Bantul	Gamping	Sentolo	Sewon	Temon	Wates
Org-matter (%)	1.37	1.51	4.39	3.32	2.97	4.67
Org-C (%)	0.79	0.86	2.54	1.93	1.72	2.71
Tot-N (%)	0.08	0.10	0.17	0.17	0.15	0.29
C/N	9.90	8.60	14.90	11.40	11.50	9.30
pH (H <sub>2</sub> O)	6.63	7.10	7.62	6.50	6.40	7.18
pH (KCl)	5.39	6.07	6.62	5.48	4.82	6.36
Textural Class	SL	SL	C	SL	L	C
% Clay (0-2 µm)	9.50	9.55	57.70	13.92	25.83	45.50
% Silt (2-50 µm)	11.86	18.11	9.83	25.83	49.12	21.04
% Sand (>50 µm)	78.64	72.34	32.47	60.25	25.05	33.66

Note: - SL = sandy loam; L = loam; C = clay  
 - Rhizosphere soil from Sedayu was not determined

#### *Effect of incubation temperature on the growth of and siderophore production by PGPR isolates*

The temperature ranges at which the *Pseudomonas* strains could grow are presented in Table 7. All PGPR isolates obtained in this experiment grew at temperature ranges from 12.5 °C to 33 °C. In contrast, *Pseudomonas aeruginosa* strains 7NSK2 and PAO286 grew at temperatures ranging from 12.5 °C to 42 °C, while *Pseudomonas fluorescens* strain ANP15 grew from 4 °C to 33 °C. Based on the differences of their ecological requirements, these PGPR isolates (44, 66, 67, 71 and 121) are most likely neither members of *Pseudomonas aeruginosa* nor *Pseudomonas fluorescens* type strains. The addition of a synthetic iron chelator EDTA (1.0 g per liter) to the growth medium (MKB) to induce iron shortage conditions is able to differentiate amongst the PGPR isolates. Under this conditions, the PGPR no. 44, 66, and 126 did not growth at 12.5 °C, but they grew better and produced siderophore at 33 °C. In contrast, the PGPR no. 67 and 71 could overcome an iron shortage at 12.5 °C, although no siderophores is produced under an iron shortage at 33 °C. The overall results demonstrate presence of ecophysiological variations or genetic diversity amongst the PGPR isolates from maize rhizospheres in Yogyakarta regions.

Table 4. Chemical composition and physicochemical properties of Patuk Soil used for pot experiment

Soil Properties	Unit Values
- Org-Matter	1.58 %
- Org-C	0.92 %
- Tot-N	0.10 %
- C/N ratio	9.20
- CEC	37.01 cmol (+) Kg <sup>-1</sup>
- pH (H <sub>2</sub> O)	6.9
- pH (KCl)	5.01
- Extractable element (NH <sub>4</sub> Ac-EDTA)	
K	136.50 mg Kg <sup>-1</sup>
Ca	1,510 mg Kg <sup>-1</sup>
Mg	522 mg Kg <sup>-1</sup>
P	1.63 mg Kg <sup>-1</sup>
- Exchangeable Al	0.07 me 100 g <sup>-1</sup>
- Field Capacity (FC)	39 %
- Texture class	Clay
Clay (0-2 µm)	54.91 %
Silt (2- 5 µm)	12.26 %
Sand (>50 µm)	32.83 %

Table 5. Effect beneficial and deleterious of rhizopseudomonad isolates on growth of maize cultivar Bisi-2 under greenhouse conditions

No.	Isolate code	Origin of isolate		Effects (%)	
		Site	Plant	Increase	Decrease
<b>A. Beneficial rhizopseudomonad (PGPR)</b>					
44	SD2.2	Sedayu	Maize	23.3	-
66	ST2.4	Sentolo	Maize	19.1	-
67	ST2.5	Sentolo	Maize	23.5	-
71	ST3.4	Sentolo	Maize	24.7	-
126	WTS.2	Wates	Maize	20.8	-
<b>B. Deleterious rhizopseudomonad</b>					
5	BT2.2	Bantul	Kolonjono Grass	-	27.0
16	GP1.1	Gamping	Cane	-	13.7
38	GP5.3	Gamping	Peanut	-	17.5
90	TM1.1	Temon	Cane	-	16.9

Table 6. Results summary of growth on some specific carbohydrates, organic acids, and amino acids/amines by plant-growth promoting rhizobacteria (PGPR)

Specific compound	Code number of PGPR isolates					
	44	66	67	71	126	<i>P. aeruginosa</i> 7NSK2
<b>A. Carbohydrates</b>						
1. D-Arabinose	-	-	-	-	-	-
2. D-Galactose	-	-	+	+	-	+
3. Lactose	-	-	-	-	-	-
4. Mannitol	+	+	+	+	-	+
5. Maltose	-	-	-	-	-	-
6. Saccharose	-	-	-	-	-	-
7. Sorbitol	-	-	-	-	-	-
<b>B. Organic acid</b>						
1. Maleate	-	-	-	-	-	-
<b>C. Amino acids/Amines</b>						
1. Betaine	-	-	-	-	-	+
2. Glycine	-	-	-	-	-	+
3. L-Leucine	+	+	-	-	+	+
4. L-Lysine	+	-	-	-	+	+
5. DL-Threonine	-	-	-	-	-	+
6. Urea	-	-	-	-	-	-

*Qualitative determination of siderophores produced by the PGPR*

Under iron deficiency, *Pseudomonas* spp. produces siderophores (Cox and Graham, 1979). The importance of siderophore production by several fluorescent *Pseudomonas* spp. either as a beneficial factor plant growth or as a virulence factor in infection was well demonstrated (Leong and Expert, 1989; Loper and Buyer, 1991). Therefore an attempt to determine siderophores by the PGPR isolates is very important for PGPR isolate characterization.

Siderophores produced by the PGPR isolates (67,71, and 126) exhibited almost the same spectral patterns whereby two peaks were observed at ca. 230.0 nm and ca. 400.0 nm (data not shown). However, All deleterious isolates (5, 16, and 90) showed distinct spectral patterns with three or four peaks (data not shown).

*Outer membrane protein analysis (SDS-Page) of 3 selected PGR strains*

Three PGPR strains of 67, 71 and 126 were selected for membrane protein analysis. Outer membrane proteins were prepared from cells (at late exponential phase) grown in LB, M9-citrate, and M9-glucose media. For all PGPR strains, distributions of proteins with different molecular weight were more expensive in LB medium. High molecular weight proteins of ca 130-kDa and ca 95 kDa were present in the PGPR strains 67 and 71, while in the PGPR strain 126 they were absent. However, two prominent bands of proteins of ca 25 kDa and ca 80 kDa were present in PGPR strain 126. The results suggest that both PGPR strains 67 and 71 are most likely two identical strains, but PGPR strain 126 is another strain. This observation supports the results of ecophysiological experiments where the PGPR strains 67 and 71 are completely identical strains (Tables 6 and 7).



Table 7. Plate assay for growth and siderophore production at different temperatures on MKB+EDTA (1 g l<sup>-1</sup>)

PGPR isolates	4 °C			12.5 °C			28 °C			33 °C			42 °C		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
44	-	-	-	+	-	-	+	+	+	+	+	+	-	-	-
66	-	-	-	+	-	-	+	+	+	+	+	+	-	-	-
67	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
71	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-
126	-	-	-	+	-	-	+	+	+	+	+	+	-	-	-
ANP15	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
7NSK2	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+
PAO286	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+

A = growth on MKB, B = growth on MKB+EDTA (1 g l<sup>-1</sup>), C = produce siderophore on A and B medium, + = growth exhibited, - = growth was not exhibited.

Reference *Pseudomonas* spp. (Anas *et al.*, 1987 a & b): ANP15 = *P. fluorescens*, 7NSK2 = *P. aeruginosa*, PAO286 = *P. aeruginosa*.

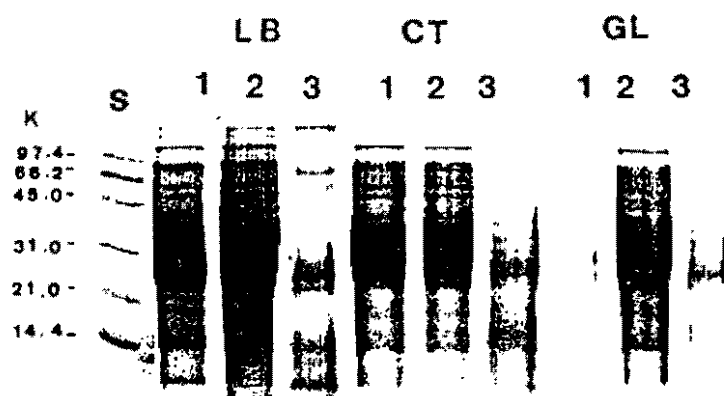


Figure 1. Outer membrane proteins: Lane S contains protein of molecular weight standard. Lanes 1 to 3 contain outer membrane proteins (opr) of the PGPR strains 67, 71 and 126, respectively. LB: Luria Broth, CT: M9-citrate, and GL: M9-glucose.

## Conclusions

One hundred thirty six rhizopseudomonads strains were successfully isolated from rhizospheres of graminaceous/cereals, grass, and legumes from 35 sites at 7 locations in Yogyakarta regions. Seventy eight isolates (57.4%) produce siderophores on solid MKB medium observed under UV light, 49 isolates (36.0%) are not pigmented, and 9 isolates (6.4%) produce non-fluorescent yellow pigment. Most isolated (97 strains or 71 %) exhibit medium to strong clear inhibition zones. Forty-eight strains had been tested for their potentiality to promote growth of maize under greenhouse conditions. Five beneficial isolates or PGPR strains increase plant dry weight from 19% to 25%. Four deleterious isolates decrease plant dry weight from 14% to 27%. The majority of isolates are neutral. Studies on eco-physiological characteristics and outer membrane protein analysis (SDS-Page) of rhizopseudomonad isolates indicate that 5 PGPR isolates can be grouped into four distinct strains. All PGPR or beneficial isolates are not likely neither members of *Pseudomonas aeruginosa* nor *Pseudomonas fluorescens*. Siderophore (microbial iron-chelating compounds) produced by beneficial or PGPR isolates exhibit almost the same spectral patterns. Two prominent peaks are identified at ca 235.0 nm and ca 400.0 nm. However, all deleterious isolates produce different spectral patterns with three or four peaks.

## Anticipated Future Research

Based on our previous research findings, the new research topics that will be conducted are (1) co-inoculation study of two or more beneficial strains on their effects on plant growth of maize on different soil types, (2) improvement of inoculation technique and inoculum formulation, aimed at (a) finding suitable technique(s) for production of rhizobacterial cells in large quantities and (b) formulating suitable carrier(s) for the inoculants, such as, peat materials, powdered coconut husk, dried compost, mineral soil, clay materials (Kaolinite, bentonite, etc.), (3) identification of environmental conditions affecting cell viability and storage lifetime of the inoculants, such as, temperature, pH, water content (drought stress), (4) compatibility studies of the inoculants with commercial fungicide(s) used as seeds treatment, (5) seed inoculation with beneficial *Pseudomonas* spp. (PGPR strains) on different crops and soil types under field conditions, (6) co-inoculation study between *Pseudomonas* spp. with other beneficial microorganisms or associations, such as, *Rhizobium*, phosphate solubilizing microorganism (mycorrhiza), *Azospirillum*, *Bacillus*, or *Azotobacter*, etc., and (7) mass production of *Pseudomonas* spp. based inoculants the improvement of the agricultural inoculation technology in Indonesia.

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## AN OVERVIEW ON ENTOMOPATHOGENIC NEMATODE (EPN) RESEARCH IN INDONESIA

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### Introduction

Entomopathogenic nematodes (EPN), referred also as insect parasite nematodes, have been recognized since the 17<sup>th</sup> century, but are only exploited as biological agents 1930's to control the Japan beetle in New Jersey, United States (Gaugler and Kaya, 1990). Their potentials were neglected by extensive use of chemical insecticides in 1940's to 1960's. After revelation of negative impacts of the chemical pesticide use, research and exploitation of EPN regained attention because they own high potentials to be used as biocontrol agents of insect pests (Buecher and Popiel, 1989).

There are two genera of EPN found almost in all over the world, *Steinernema* and *Heterorhabditis* (Poinar, 1990). The genus *Steinernema* belongs to the family of Steinernematidae from order Rhabditida. *Heterorhabditis* included in the family of Heterorhabditidae (order Rhabditida) is new genus and family found by Poinar. Genera *Steinernema* and *Heterorhabditis* have more pathogenic species to insect than other genera. Up to now there are 16 pathogenic species of *Steinernema* and six species of *Heterorhabditis* which have been identified.

Pathogenicity of EPN to insect happens through mutualistic symbiosis with pathogenic bacteria of *Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis* (Forst *et al.*, 1997). Symbiosis complex of EPN - bacteria is very ideal to be developed as biological agents of pest insects, because they are active, own high virulence, have wide host range, grow on artificial media, easy to apply, save for the environment, and compatible with some types of chemical pesticides (Gaugler and Kaya, 1990). Bacterial cells are brought into the infective juvenile (third IJ) of the EPN. The EPN bacteria are unable to freely live in nature. Infection process only happens through penetration of EPN into insect.

In some countries, symbiosis complex between bacteria - EPN has been exploited as biocontrol agents for insect pests (Friedman, 1990). Some commercial biopesticide products using EPN as active ingredient have also been marketed. In an effort to develop potency of Indonesian indigenous EPN, Indonesian Agricultural Biotechnology and Genetic Resources Research Institute (IABGRRRI, formerly Balitan), Bogor, through research cooperation with University of Wales, Cardiff, England has explored EPN from all over Indonesia. Potential strains of EPN are being developed and formulated as effective and environmentally friendly biopesticides. At present, research has reached *in vitro* mass production using artificial media and granulate formulation with alginate. Dissemination of conventional mass production technology that is easy to be adopted by farmers has also been conducted through cooperation with Balai Peramalan Hama Tanaman Pangan dan Hortikulutura (BPHTPH) and Balai Penerapan dan Pengkajian Teknologi Pertanian (BPPTP). This paper reviews research on EPN conducted at the IABGRRRI since 1993.

### EPN Collection

First step of research is exploration to collect indigenous Indonesian strains of EPN. Locations of survey are Java (Cisolok, Pelabuhan Ratu, Serang), Bali (Candidasa, Surabrata, Medewi, Basangkasa, Petingan), Ambon (Latuhalat, Toisapu, Natsepa, Waai, Liang), Seram (Kairatu Ferry, Kamal, Hatu Sua), and Sulawesi. From each location, sandy soil or soil with high porosity having appropriate properties for EPN habitat were taken. In the laboratory, sample was placed in bottles, infected with Hongkong caterpillar (*Tenebrio molitor*) or *Galleria melonella* for trapping EPN. After

incubated for five days, existing EPN infected the larva. Symptoms of larva infected by *Heterorhabditis* differ from those of larva infected by *Steinernema*. Dead caterpillars infected by *Steinernema* are young tan, while those infected by *Heterorhabditis* are reddish, dark tan.

In this exploration, 23 strains of *Steinernema* and 24 strains of *Heterorhabditis* were collected by. PCR analysis indicated that four strains of *Heterorhabditis* were identified as *H. indicus*, while strains of *Steinernema* have not been identified, but they were anticipated to consist of two different types by RFLP analysis.

### EPN Mass Production

Mass production of EPN can be conducted through two ways, by *in vivo* using Hongkong caterpillar or bamboo caterpillar, and by *in vitro* using artificial media.

Under *in vivo* mass production method, EPN is multiplied in Hongkong caterpillar (*T. molitor*) or honey caterpillar (*Galleria mellonella*) according to the method of Woodring and Kaya. EPN is grown on filter paper in petri dish (diameter 9 cm). As much as 1.4 ml of EPN suspension containing 100 - 200 IJ/ml is dripped into petri dish, before 30 caterpillars of *T. molitor* are infested into the dish. After 5 - 7 days of incubation, infected larvae are transferred to a trap in the form of a plastic box (30 x 20 x 4 cm) with aluminium layered by muslin cloth. Water is added into the plastic box till the height of 0,5 cm. IJ of EPN will come out and they can be harvested 12 to 20 days after inoculation. Before use, IJ can be kept in aerated water or in sponge incubated at room temperature.

Production of *Heterorhabditis* in Hongkong caterpillar can reach 0,6 - 0,76 x 10<sup>6</sup> IJ/gm (Fallon *et al.*, 1995). Production of EPN is influenced by inoculum concentration per larva. According to Chaerani *et al.* (2000), the most optimal inoculum concentration is 50 - 200 IJ/larva. After inoculation, IJ start to emerge on the day 9 until day 30. Maximal production occurs 14 days after inoculation, reaching 0,12 x 10<sup>6</sup> IJ/larva. The best production happens in honey caterpillar, *G. mellonella*, that is 1,6 x 10<sup>6</sup> IJ/gm. Production of *Steinernema* in Hongkong caterpillar reaches 0,88 x 10<sup>6</sup> IJ/gm and in *G. mellonella* reaches 1,4 x 10<sup>6</sup> IJ/gm.

*In vitro* propagation using artificial media is in fact more difficult and complicated because it depends on primary phase of the symbiont bacterium (Akhurst, 1980), but is more efficient for commercial or big scale production. The media consist of substances with high proteins such as homogenate intestine, liver extract, yeast extract, egg, peptone, soy flour, etc. (Bedding, 1984; Chaerani *et al.*, 2000; Budihardjo *et al.*, 2002). Propagation can be conducted in solid or liquid media. Solid media with sponge are most commonly used because as their porosity is suitable for EPN habitat. Nutrition is soaked into sponge with ratio of 12.5: 1 (media: sponge, W/W) (Bedding, 1984). The sponge is put into heat-resistant plastic or bottle, and then sterilized. After the medium is cool, the primary bacterium is inoculated into the media. Bacterium is allowed to multiply for 2 - 3 days before inoculation by as much as 1000 - 2000 IJ/bottle volume 500 ml. EPN can be harvested two weeks later. Every gram of sponge media can yield 100 - 200 thousand IJs.

In propagation with liquid media, media are put input fermentor with capacities of 1500 ml. The media are then sterilized in an autoclave for 30 minutes at temperature of 121 °C. After chilling, media are inoculated with 4.2 x 10<sup>7</sup> cells/ml of bacteria of primary phase in NaCl 0,5%. Two days later, as many as 105 IJ/ml EPN are inoculated into the media. Production of *Heterorhabditis* by this method can reach 0,087 - 0,096 x 10<sup>6</sup> IJ/gm media (Chaerani *et al.*, 2000). This result is lower compared to result obtained in Maynooth, Ireland, which can reach 0,7 x 10<sup>6</sup> IJ/gr media (Fallon *et al.*, 1995). Expenses required to produce one million of IJs is Rp 488,00 based on calculation in the year of 1995.

In the same way, production of *Steinernema* can reach 0,11 - 0,16 x 10<sup>6</sup> IJ/gr media. This result is also lower compared to result obtained in Maynooth, which can reach 0,4 x 10<sup>6</sup> IJ/gr media. This difference is anticipated due to different ability of each strain to multiply or nutrition quality. *In vitro* methods of mass production require further development for larger scale production.

### Development of EPN Biopesticide Formulation

In developing EPN as biopesticides, besides mass production problem, formulation and storage techniques require attention, because EPN as organisms have limited tolerance levels. EPN do not own dormant phase. Growth stadium of EPN that can be used as an active substance for development of biopesticide is IJ stadium, because this stadium survives longest when the host is absent.

To investigate IJ viability in storage without decreasing its pathogenicity, storage methods at various temperature levels (9 °C, 11 °C, 13 °C, and 15 °C) have been studied using various substances (water, Ringer solution, formalin, sea water, glycerol) and media. Results of this research indicate that storage of IJ in Ringer solution at temperature 15 °C is the best storage condition. Viability of IJ still reaches more than 60% after eight-depository weeks. Ringer solution has salinity almost similar to insect haemolymph. Carrier media that are good for storage are alginate or sponge compared to kaolin, active charcoal or tapioca.

### Bioefficacy of EPN and Their Symbiont Bacteria

Strains of EPN collected have been tested for their effectiveness to some types of pest insects, either in laboratory or in the field, such as Hongkong caterpillar (*T. molitor*), "grayak" caterpillar (*S. litura*), "boleng" pest of sweet potato (*Cylas formicarius*), potato leaf borer, and some other insets such as *O. furnacalis*.

Results of bioefficacy test in the greenhouse indicate that with concentration of 60.000 IJ/pot (diam. 30 cm), *Heterorhabditis* is able to depress population of *C. formicarius* and potato leaf borer until 66% and 70%, respectively (Chaerani and Waluyo, 1996; Yulensri, 2001). Effectiveness of *Steinernema* in decreasing population of yellow rice stem borer (*Scirpophaga incertulas*) with concentration of 20.000 IJ/bunch reaches 86%, but effectiveness of *Heterorhabditis* to *S. innotata* with concentration of 20.000 IJ/ bunch only reaches 35% (Fallon et al., 1995).

Field experiment in Sukamandi shows that application of *Heterorhabditis* with concentration of 20.000 IJ/ml can depress population of *S. innotata* until 67%. But subsequent experiment with concentration of 5.000 IJ/ml results in the death of *S. innotata* of only by 39%. The same death level is also shown in application of *Steinernema* with concentration of 10.000 IJ/ml (Fallon et al., 1995). This matter indicates that Indonesian population of *Heterorhabditis* and *Steinernema* has enough potential to be developed as biocontrol agents of insect pests. Excellence of EPN as biocontrol agents is because EPN IJ is active to find host target, has wide host range, has high virulence that can kill host insect in less than 48 hours.

In addition to EPN, bacteria of *Photorhabdus* and *Xenorhabdus*, which symbiotically live with EPN, are also very pathogenic to insect. Results of bioassay test by injection technique into insect haemolymph using microsyringe indicate that with concentration of 40 cell/larva, both bacteria are able to cause 80% mortality of *T. molitor* (Samudra et al., 2002). High pathogenicity of the bacteria is because of secretion of certain toxins. Toxicity of the toxin is also known to have wide host range. In the next research program, gene coding for toxin known as polypeptide with molecular weight of between 66 - 300 kDa, will become a target of cloning program for development of biopesticide and transgenic plants.

### Future Perspectives

Two genera of EPN, *Heterorhabditis* and *Steinernema*, are collected from some locations in Indonesia and which are found in almost all places. This means that both EPN have been widely spread in Indonesia. Some species of pest insects are very susceptible to EPN of Indonesia population, either in the laboratory or in the field. With this potential, EPN and their symbiotic bacteria deserve more attention, especially in the frame of the conservation of below ground biodiversity program in Indonesia.

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## WHAT IS THE ROLE OF BELOWGROUND BIODIVERSITY IN ESTATE CROP FARMING SYSTEMS?

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### Introduction

In Indonesian economy, estate crops play a key role by contributing significant amount of export of non-gas-oil commodities and the foreign currency income. Besides, most of the 5 million hectares of the estate crops belong to small holders. High productivity of this commodity should be supported by proper soil fertility. On the other hand, it is crucial to maintain soil fertility of estate crops in Indonesia due to the frequently found nutrient deficient soils.

Use of fertilizers and pesticides that is a significant expenditure to overcome nutrient and pest problems in estates is a common practice nowadays. By economical considerations, there should be efforts to reduce the use of those fertilizers and pesticides. Furthermore, taking into account the amount of high quality raw materials for fertilizers and increase in cost of the unrenewable energy sources and to assist sustainable agriculture, the effort to minimize the use of inorganic fertilizers and pesticides should be connected with development of energy saved agriculture by managing belowground biodiversity. As important living agents in nature, soil microorganisms may positively or negatively affect growth and production of estate crops.

Beneficial soil microorganisms as parts of belowground biodiversity are particularly important for tropical estates in which the availability of nutrients for plants is commonly low. Therefore, there is a need to enhance both the capacity of plants to explore nutrients sources and the availability of nutrients and to improve soil structure, to increase plant tolerance to environmental stress and to soil borne pathogens by managing the belowground biodiversity.

This paper aims to review Indonesian published reports of the management and functions of some belowground microorganisms in the estate crops farming systems, involving coffee, cocoa, oil palm, rubber, tea and sugarcane plantations in Indonesia. This review will discuss the role of belowground organisms under each estate crop ecosystem.

### Belowground Organisms

Beneficial and harmful plant-microbe interactions in the belowground are primary determinants of plant health and soil fertility. Several groups of organisms are discussed in this review. Arbuscular mycorrhizal fungi (AMF), phosphorus solubilizing bacteria, *Rhizobium*, non-symbiotic N-fixing bacteria, collembola and the biocontrol agent *Trichoderma* spp. are the beneficial organisms to be discussed, while the harmful organisms are parasitic nematodes and pathogens such as *Rigidoporus* spp., *Ganoderma* spp.

AMF are the most important microbial symbionts for the majority of plants, particularly under conditions of P-limitation. They influence aboveground productivity in estate crop farming systems. The mycorrhizal symbiosis is a keystone to the productivity and diversity of natural plant ecosystems and it is rare to find a situation where AM do not have a significant ecological presence. *Rhizobium*, P-solubilizing bacteria and non-symbiotic N-fixing bacteria are the other microorganisms taking parts in biogeochemical cycling of both inorganic and organic nutrients in the soil and in the maintenance of soil quality.

*Pratylenchus coffeae* is a serious parasitic nematode both on arabica and robusta coffee, while *Ganoderma* spp. and *Rigidoporus* spp. are the most economically important pathogen of oil palm and rubber, respectively. The affected trees generally die and fall prematurely reducing stand

population and the yield of plantation. In most cases the diseases are difficult to manage and the infected plants usually show symptoms of infection only after a large portion of its base has been destroyed by the pathogen.

## Cocoa

### *Arbuscular mycorrhizal fungi*

#### Ecology

A new species of *Acaulospora*, *A. walkeri*, was found associated with cocoa plants in Java and Bali (Kramadibrata & Hedger, 1990). Meanwhile, from the rhizosphere of cocoa in West Java, *A. delicata*, *A. walkeri*, *Glomus cf. citricolum*, *G. fuegianum*, *G. rubiforme*, *G. globisporum*, *A. foveata* and three other unidentified species of *Glomus* were found (Widiastuti & Kramadibrata, 1992).

Different location and rhizosphere resulted in diversity in species and population of AMF (Widiastuti & Kramadibrata, 1992). In that study, it was found that in *Imperata* and maize rhizosphere population of AMF spores was higher than that of cocoa. Soil of research site (Widiastuti & Kramadibrata, 1992) was dominated by clay fraction which is suitable for development of *Glomus* spores, whereas sandy soil is suitable for *Gigaspora* species. Baon *et al.* (1996) found that cocoa roots colonized by AMF did not affect aggregate stability of sandy soil reflected from dispersion ratio values.

#### Growth and nutrient

Colonization by AMF affected growth of cocoa (Baon, 1994; 1996a-b; Lucia, 1994; Muslimin, 1994). Response of cocoa growth to AMF infection may reach more than 100% (Baon, 1994). Cocoa infected with AMF, *Gigaspora margarita*, had higher content of foliar P than nonmycorrhizal cocoa, although there was a variation among cocoa clones (Baon, 1995). On the other hand, uptake of less mobile micronutrients, Cu and Zn, was also enhanced by AMF infection of cocoa (Baon, 1994).

### *Collembola*

Agroecosystem of cocoa plantation, which also involves dry cocoa leaves as mulch, provides a favorable condition for development of natural enemies of cocoa pests. Collembola is believed to take part in decomposition of organic matter. High population of collembola enhanced the population of parasitoid of cocoa pests. Cocoa plantation with thicker layer of cocoa leaf mulch tended to have high population of collembola (Sullystowati *et al.*, 2003). Application of pesticide and dry climate reduced the population.

## Coffee

### *Arbuscular mycorrhizal fungi*

Population of AMF spores in two coffee cultivars was similar, but decreased by application of P fertilizer (Baon, 1995).

Without mycorrhizal infection growth of arabica coffee was stunted. AMF *G. margarita* effectively increased growth of coffee seedlings (Wibawa & Baon, 1990; Baon, 1995; 1996a-b) and plantlets (Winarsih & Baon, 1999). On the other hand, Hastuti (1998) found that the AMF *Gigaspora rosea* tended to reduce growth of robusta coffee.

Response of coffee to AMF infection varied according to coffee variety (Baon, 1996a-b) and type of AMF symbionts. Robusta coffee cv. BP 42 x BP 358 responded more significantly to AMF than cv. BP 350 x BP 42. It was found that less efficient coffee genotype is more responsive to AMF infection (Baon, 1995). Hanapiah (1997) reported that arabica coffee height increased significantly when infected by *Gigaspora margarita*, but not by *Glomus manihotis*. However, combination of the two increased more significantly the growth of arabica coffee.

#### *Arbuscular mycorrhizal fungi and parasitic nematodes*

Parasitic nematodes as part of belowground biodiversity are a difficult organism to control because they have a wide variation of hosts, high ability to survive in unfavorable condition. Some works have been carried out to investigate the role of AMF associated with arabica coffee and robusta coffee (Baon & Wiryadiputra, 1995; 2001) on their tolerance to the infestation of parasitic nematodes. Colonization of AMF *Gigaspora margarita* on robusta coffee resulted in similar growth and yield potency compared to nematicide. However, on arabica coffee the parameters were better in plants treated with nematicide compared to ones infected with *G. margarita*. *G. margarita* reduced the population of *Pratylenchus coffeae* but was not effective to *Rotylenchulus reniformis*. Robusta coffee was more resistance to *P. coffeae* than arabica coffee (Baon & Wiryadiputra, 2001). Coffee plants colonized with an AMF *G. margarita* were able to reduce the negative effect of the infestation of *P. coffeae* (Baon & Wiryadiputra, 1995).

#### *Shade trees, Rhizobium and AMF*

A number of estate crops, such as coffee, need shade trees for their growth and production which function in reducing light intensity, wind hazard and supplying organic matter, besides providing good condition for belowground microorganisms. Colonization of shade trees by AM fungi increased growth of *Gliricidia maculata* and particularly *Moghania macrophylla* but not *Sesbania grandiflora* (Baon, & Wibawa, 1994). Highly infected shade trees may benefit the main crops by transferring the infection points and nutrients due to the presence of hyphal network. There was a trend that growth of shade trees was more significant when AMF and *Rhizobium* colonized roots of the plants. The density of mycorrhizal spores in *S. grandiflora* was lower than those observed in *G. maculata* and *M. macrophylla*.

#### **Oil Palm**

##### *Arbuscular mycorrhizal fungi*

##### Ecology

Puspa & Suwandi (1990) found in oil palm plantations in North Sumatera, six species of AM fungi, three *Glomus* spp. and one each for *Acaulospora* sp., *Gigaspora* sp. and *Scutellospora* sp. While from rhizosphere of oilpalm roots in West Java, it was found that AMF colonized only cortex tissue and formed vesicles, arbuscules and coils. It is possible that more than one species of *Glomus* infected the oil palm roots (Widiastuti & Kramadibrata, 1993). From the soil and root samples, 11 species were identified, i.e. *Acaulospora joveata*, *A. mellea*, *A. scrobiculata*, *A. tuberculata*, *Glomus fuegianum*, *G. globosporum*, *G. rubiforme*, *G. sianosum* and three unidentified species of *Glomus*.

##### Growth and nutrition

Growth and nutrient uptake of tissue culture derived oil palm seedlings were improved by AMF colonization. Oil palm infected with *Entrophospora columbiana* had fast growth (Widiastuti & Tahardi, 1993). In other study by Schultz *et al.* (1999), it was found that 10 of eleven AMF isolates increased survival rates of oil palm plantlets, except one isolate. Two species of *Acaulospora* were the most suitable and effective to increase survival rates of oil palm plantlets. On the other hand, Heningtyas (1997) found that five species of AM fungi (3 *Glomus* spp., *Glomus manihotis*, *Acaulospora* sp. and mixture of them) did not affect growth of oil palm.

##### *Ganoderma* spp.

Basal stem rot caused by pathogenic fungus *Ganoderma boninense* is a very important disease of oil palm. Several genera of soil fungi, which are antagonistic to *G. boninense*, have been isolated from soil of several oil palm plantations, and the very potential one was *Trichoderma harzianum* (Abadi, 1987). High population of *T. harzianum* in soil could suppress growth and viability of

*G. boninense* (Purba et al., 1996). Inoculum potential of *G. boninense* was higher in root tissue than in basal stem tissue.

## Rubber

### Arbuscular mycorrhizal fungi

Colonization of rubber plant roots by AMF (*G. fasciculatum* and two isolates) increased growth and phosphorus (P) uptake of the plants, and the effects varied depending on the species of the fungi and growth environment (Istianto et al., 1995). Synergistic effect was found when the rubber plants were also fertilized with rock phosphate. In other work it was found that *Gigaspora margarita* did not influence growth of rubber plants (Susanto, 1994).

### Rhizobium, AMF and cover crops

Legume cover crops are widely used in rubber plantations. Co-colonization between *Rhizobium* and AMF *Glomus fasciculatum* in leguminous cover crops, *Calopogonium caeruleum* and *Pueraria phaseoloides*, could overcome the competition effect between the two cover crops in N and P uptake and the rubber trees (Nasution & Istianto, 1995). Infection of *Rhizobium* and AMF on cover crops reduced non-productive period of rubber trees.

### Pathogenic fungi

White root disease is the most important disease in rubber, which is caused by *Rigidoporus microporus*. Two soil saprophytic fungi, *Trichoderma viride* and *Gliocladium* sp., showed the capacity to reduce the infestation of *R. microporus* (Basuki & Sinulingga, 1996). As facultative parasite, *R. microporus* can live both on living and decaying root tissues. Sinulingga (1996) reported that *Trichoderma koningii* was able to suppress fruit bodies and rhizomorph of *R. lignosus*, although in other locations contrast results were obtained due to unfavorable environmental factors.

In different study, Purba et al. (1995) found that population of several soil microflora, i.e. bacteria, fungi and *Fusarium* varied in different soil. Dominant *Fusarium* species was *F. oxysporum* which is non-pathogenic to oil palm. All soils studied were receptive to pathogenic strains, but they varied in their receptiveness. There was still no clear relationship between the level of receptiveness and microflora density to fusariosis.

## Tea

### Arbuscular mycorrhizal fungi

Total and available P in soil, and its uptake by tea plants were not affected by colonization by *Glomus fasciculatus* (Salim et al., 1999).

### Phosphate solubilizing microorganisms

From rhizosphere of tea plants, Rachmiati et al. (1995) found 12 distinct isolates of P-solubilizing bacteria, which had different capacity in solubilizing less soluble phosphate. The selected isolates were further studied and the results showed that the presence of the P-solubilizing bacteria did not influence soil soluble P, foliar P and leaf dry weight (Rachmiati & Salim, 1998).

## Sugarcane

### Arbuscular mycorrhizal fungi

Nuhamara (1980) found a specific kind of spores in rhizosphere of many sugarcane plants in East Java. From an experiment conducted in an ultisol soil, it was found that sugarcane infected with AMF *Glomus fasciculatus* produced higher dry weight of sugarcane (Simoen & Windiharto, 1988). Better growth of sugarcane was also found by Adinurani et al. (1999) when sugarcane was colonized

by AMF in two locations. Sugarcane yield, amount of crystal and the out-turn were also increased by the mycorrhizal infection.

#### *Non-symbiotic N-fixing bacteria*

Presence of free-living N-fixing bacteria is based on the fact that sugarcane can be grown for more than 30 years without N fertilizer application. Widayati (1996) showed that N-fixing bacteria can live in association with sugarcane cuttings and its distribution can reach until inner side of stalk as indicated by the presence of nitrogenase activity in cane juice.

Ten isolates of N-fixing bacteria were positively identified as diazotrophic bacteria as expressed by activity of acetylene reduction (Widayati, 1998). From the selected isolates, five isolates demonstrated the presence of phytohormon, although only two isolates from the five isolates were derived from rhizosphere.

#### *Phosphate solubilizing microorganisms*

Isolates of *Pseudomonas putida* LP.112-1, LP. 312-1 and L27A4A1 were able to solubilize less-soluble phosphates and therefore increased fertilizer efficiency 10-20% of sugarcane (Widayati & Premono, 1999). This ability was related to the capacity of the microorganisms in producing organic acids and phytohormons.

#### Conclusions

This review has highlighted the practical situations where various microorganisms, both beneficial and harmful, of belowground under plantation crops have a significant impact in restoring or maintaining plant health, aboveground productivity and soil fertility. They are not unusual situations, but are commonplace throughout Indonesian estate crop farming systems. Although they probably represent the most significant above- and belowground relationship, the organisms discussed in this review are just one facet of the complex belowground interactions that occur in the estate farming ecosystems. It is vital that scientists pay them due attention in any schemes to increase, restore or manage plant health and soil fertility.

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**ECOLOGICAL SIGNIFICANCE OF ROOT ARCHITECTURE  
OF DROUGHT RESISTANT PLANTS IN IMPROVING  
DIVERSITY AND SERVICES OF BELOWGROUND ORGANISMS**

**A case study of promoting a synchrony of organic fertilizer  
from decomposition of local plant diversity  
to sustain limestone upland soil fertility of the Brantas River Watershed**

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**Introduction**

Dryland farming in East Java, especially in the upstream area of the Brantas River Watershed, has not paid attention to conservation aspects, and distribution of marginal land is getting wider. Soil Survey in 1988 found that some of the Brantas River Watershed is in the form of limed dry land with shallow soil solum, inclination 5-60 %, low soil fertility and low soil productivity. Maize production is less than 1 ton ha<sup>-1</sup>, whereas cassava is less than 10 ton ha<sup>-1</sup> (Utomo, 1989). Inorganic fertilizer is less efficient because fertilizer can be easily leached by rain. Low content of soil organic matter (< 2%) has caused low soil buffering capacities. To support growth of maize and cassava, this marginal land requires very high organic matter inputs, minimal 10 ton ha<sup>-1</sup> which is difficult to be fulfilled by farmers.

In addition, in some marginal lands, soil condition is so bad that the soil is unable to produce enough biomass to be returned to the soil. This difficulty in fact can be overcome by, for example improving sources of organic matter although crop production is still less than optimal. Another problem is synchronization between release period of nutrition from organic matter and crop requirement of nutrition. Organic matter originated from rest of Leguminosae only releases nutrition of about 20-45 % from its contents (Handayanto *et al.*, 1994). In one crop season, from that amount, only about 30 % that can be used by crop (Handayanto *et al.*, 1997). If nutrition supply is not in synchrony with time, nutrition availability, and crop requirement of nutrition, then deficiency or excess of nutrition will happen although total amount of supply is equal to requirement (Handayanto, 1999). The synchronization is determined by the speed of decomposition and mineralization of organic matter.

N concentration, C/N ratio, lignin and polyphenol concentration are factors influencing decomposition and mineralization of organic matter (Handayanto *et al.*, 1997). Besides organic matter quality, processing of organic matter can also influence the synchronization. Decomposition and mineralization of organic matter placed as mulch on soil surface run slower than if it is dipped into soil. Sustainability of the limed soil fertility through optimal development of local flora diversity is needed.

Results of previous studies indicate that 260 C<sub>1</sub>-C<sub>3</sub> local flora that have been adapted to local conditions can be potentially exploited as permanent land cover vegetations (Arisoesilaningsih *et al.*, 2001). In addition, Leguminosae has been recognized to have ability to improve N status and soil biology. Some wild or domesticated species grow quickly than others, for example *Dalbergia latifolia*, *Sesbania sesban*, *Lantana camara*, *Tithonia diversifolia*, *Cajanus cajan*, *Phaseolus lunatus*, *P. vulgaris*, *Mucuna* spp., etc. Conversion of sunlight energy in plant cells improves accumulation of organic matter available to higher trophics. Accumulation of this organic C will increase various biological activities, improve soil properties, and protect soil from by outside troubled influences such as erosion, leaching, condensation, etc. Crops with high productivity, water efficiency, light efficiency, nutritive, and energetic are expected to become base of selection and model system of polyculture or mixed cropping.

Based on the above-mentioned background, this research aims to determine productivity, water efficiency, light efficiency, C, N, P contents, and calorie of 14 Leguminosae species growing in



limy marginal lands of the Brantas River Watershed. Process and outcome of this research will touch poor farmers and society *in situ*. Success of ecosystem and soil remediation is also expected to lessen fertilizer and pesticide use, and avoid land exploitation and rural development that are not environmentally friendly. This research is also expected to (1) contribute to scientific advancement and environmental protection technology, (2) disclose roles of genetic diversity of plants adapted to limy dry area, (3) preserve Indonesian flora for stability of ecosystem productivity, and (4) obtain more efficient soil conservation techniques so that the land can be more productive all year long.

## Methods

### *Characterization of local plants and effect of multicropping maize*

Leguminosae used in this research consisted of 14 species (Table 1). Eight of them are familiar to and grown by local farmers, the other six species represent wild plants growing in unattended lands or roadside.

Table 1. Diversity of Leguminosae used in this research

Group	No.	Latin name	Local name	Life form
Crop	1.	<i>Cajanus cajan</i>	Gudhe	entangle, annual
	2.	<i>Canavalia gladiata</i>	Koro pedang	herb, annual
	3.	<i>Dolichos lablab</i>	Koro uceng	creep, biannual
	4.	<i>Mucuna pruriens var. utilis</i>	Benguk	creep, annual
	5.	<i>Phaseolus lunatus</i>	Koro krupuk	creep, biannual
	6.	<i>Phaseolus vulgaris</i>	Koro ebek	creep, annual
	7.	<i>Psophocarpus tetragonolobus</i>	Kecipir	creep, annual
	8.	<i>Vigna sinensis</i>	Kacang tunggak	creep, annual
Wild plant	9.	<i>Cassia hirsuta</i>		bush, perennial
	10.	<i>Cassia mimosoides</i>		herb, perennial
	11.	<i>Centrosema pubescens</i>		herb, perennial
	12.	<i>Crotalaria usaramoensis</i>	Orok-orok	herb, annual
	13.	<i>Flemingia aspera</i>		bush, perennial
	14.	<i>Mimosa somian</i>		bush, perennial

Water efficiency experiment was conducted at the glass house, Department of Biology, FMIPA, Brawijaya University. Crop was grown in poly bag containing soil of limy marginal land mixed with animal manure and NPK. Poly bags were sprinkled routinely so that the soil is in the field capacity condition. Water consumption was measured gravimetrically once a week. Stoma opening was observed in the morning, at noon and in the evening near the harvest time (120 day after planting). Micro climate (light intensity, temperature, and air relative humidity) and soil humidity were perceived simultaneously with stomata measurement. At the harvest time, biomasses of root and shoot weighted. Water efficiency was determined by comparing plant productivity with accumulation of water consumption (Larcher, 1995).

Vegetation covering and polyculture system were conducted with two plots: monoculture maize and polyculture maize (mix of maize with Leguminosae) in a farmer's land in the Pagak Village, Pagak Subdistrict, Malang. This experiment was also used to determine productivity and light efficiency. Each species of Leguminosae was grown at random for six months between maize of local varieties. Weed, natural enemy and pest were not disturbed with pesticides. Light efficiency was determined by comparing productivity with means of light intensity received during growing season. Effect of vegetation covering and root system were perceived through determination of microbial community, soil fauna diversity and aerial arthropods, and soil abiotic character (C, N, pH and soil humidity).

*Decomposition of more efficient organic matter as source of P fertilizer*

Local flora investigated as P sources included *Acacia villosa*, *Ageratum conyzoides*, *A. houstonianum*, *Gliricidia sepium*, *Lantana camara*, *Pennisetum purpureum*, *Tectona grandis*, *Tithonia diversifolia* and *Zea mays*. The local plants were packed into the Entisol soil that has been fertilized with P and K. Decomposition rate and mineralized P were analyzed periodically. Simultaneously, organic matter was mixed with soil, and two weeks later maize was grown. P efficiency by maize was measured according to the method explained by Pratikno *et al.* (2002).

*Decomposition of organic matter to produce synchronic N fertilizer*

Quality of local flora biomass was examined by determining total contents of C, N, and P, polyphenol and lignin. Local flora examined as N source were *A. villosa*, *M. somian*, *Cassia mimosoides*, *C. pubescens*, *F. congesta*, *M. pruriens* var. *utilis*, *P. lunatus* and *P. tetragonolobus*. The organic matter was sinked into the Entisol soil that has been fertilized with P and K. Two weeks after the mixing, maize was cultivated. Soil N content and wide and high of maize leaf were measured periodically. At the age of eight weeks, maize was harvested and content of absorbed N was analyzed. Percentage of N recovery was determined according to the method explained by Sunaryo and Handayanto (2002). Similar experiment was also conducted in the field until maize was harvested.

**Results and Discussion***Water efficiency*

From the glasshouse experiments, it was known that plant growth experienced modification due to glasshouse conditions (daytime temperature could reach 37 °C, light intensity was low) and limited size of polybag. The Growth problem was seen at *C. gladiata*, *D. lablab* and *M. pruriens utilis*, while *C. pubescens*, *C. hirsuta* and *F. aspera* showed better growth than in field. Limitation of method of water consumption determination enabled the green house data can be used as considerant.

Productivity of *M. pruriens utilis*, *P. lunatus*, *C. gladiata* and *C. pubescens* reached 12 - 17 g bl<sup>-1</sup>, while *C. usaramoensis* and *F. aspera* only reached 8-10 g bl<sup>-1</sup>. Table 2 indicated that *D. lablab* consumed most water, 7,9 kg bl<sup>-1</sup>, while *P. lunatus* and *F. aspera* consumed relatively small amount of water (3,6 - 5,2 kg bl<sup>-1</sup>). In this research, *M. pruriens utilis*, *C. gladiata*, *P. lunatus* and *F. aspera* own water efficiency of (2,3 - 2,4 g kg<sup>-1</sup>), higher than *D. lablab* and *C. usaramoensis* (1,4 - 1,8 g kg<sup>-1</sup>).

Thus, *M. pruriens utilis* and *P. lunatus*, which are planted by local farmers with high water efficiency, can be exploited as green manure producer. Short-lived *M. pruriens utilis* can be planted two times a year and is included into plants with rapid carbon gain (investment type) (Larcher, 1995). Crop grows fast vegetatively to produce leaf biomass. *P. lunatus* can be planted in two seasons (biannual). This crop generally grows slowly and own conservative budget. In the field, *P. lunatus* keeps forming new leaves, flowering and producing nuts at the 2002 dry season. Besides drought tolerance, *P. lunatus* is also water efficient. It is supposed that *P. lunatus* is equipped with certain physiological and morphological adaptation. There is a possibility that the crop develops deep root system.

Table 2. Water consumption and water efficiency of some local flora used

Crop	Water consumption (kg bl <sup>-1</sup> )	Water efficiency (g kg <sup>-1</sup> H <sub>2</sub> O)
<i>C. gladiata</i>	5,5 ± 0,6	2,4 ± 0,6
<i>M. pruriens utilis</i>	6,9 ± 0,6	2,4 ± 0,3
<i>P. lunatus</i>	5,2 ± 0,6	2,4 ± 0,1
<i>F. aspera</i>	3,6 ± 0,4	2,3 ± 0,3
<i>C. pubescens</i>	6,7 ± 0,7	2,2 ± 0,1
<i>C. usaramoensis</i>	5,5 ± 0,6	1,8 ± 0,5
<i>D. lablab</i>	7,9 ± 0,4	1,4 ± 0,1

### Organic matter productivity and light efficiency

Except *C. usaramoensis*, *V. sinensis* and *C. gladiata*, other crops aged 75 after planting were defoliated (reduction half of existing leaf abundance). *C. gladiata* and *C. usaramoensis* were not defoliated due to low leaf abundance. This was intended to give organic biomass for green manure at the same season. Four Leguminosae tested exhibited good tolerance to defoliation (Figure 1).

This defoliation is beneficiary because besides green manure obtained, fruit production was not troubled. Soil cover plants got more light intensity and grew better. In short range, it was seen that crop mulch experiencing defoliation was less. However, at harvest the (150 days after planting), defoliation effect were not seen and the entirety growth tended to be equal. This indicates that crops defoliated at the vegetative growth log phase, and when soil availability is not a constraint, recover quickly.

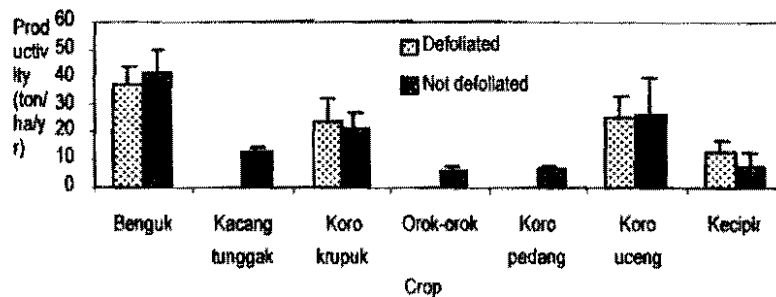


Figure 1. Organic matter productivity of local flora due to defoliation.

Note: *C. usaramoensis* and *C. gladiata* were not defoliated, lines at the bars show relative deviation ( $\alpha = 0,05$ ).

*M. pruriens utilis*, *D. lablab*, *P. lunatus*, *P. tetragonolobus* and *V. sinensis* produced high total productivity of organic matter ( $> 10$  ton dry weight  $ha^{-1} yr^{-1}$ ). *M. pruriens utilis*, *P. lunatus* and *D. lablab* even yielded organic matter of 20 - 40 ton dry weight  $ha^{-1} yr^{-1}$  or equivalent with 80 - 160 ton fresh weight  $ha^{-1} yr^{-1}$ . As many as 50 % of the productivity can be consumed by farmers as vegetables or tempe, then the organic matter is transferred from the agroecosystem. According to Agus (2002), besides loss through erosion, soil nutrients can be disappeared or unavailable for crops because of harvesting, fixation, leaching or N volatilization. But, considering that organic matter required by local farmers reaches at least 10 ton fresh weight  $ha^{-1} yr^{-1}$ , *M. pruriens utilis*, *P. lunatus* and *D. lablab* provide enough organic matter, either through mulch or crop residue at harvest time. To support healthy and sustainable agroecosystem, these Leguminosae can be planted as fence crops or mixing crops to increase plant diversity, while their coverage is good to protect soil, prevent erosion, and improve soil structure (Agus, 2002) and soil biota habitat (Sari *et al.*, 2002 and Siswanto *et al.*, 2002). In addition, diversity at producer level will be followed by improvement of diversity of herbivore taxa (pest or non-pest) and also predator/parasitoid of natural enemies (Arisoeslaningsih *et al.*, 2002).

*M. pruriens utilis*, *P. lunatus* and *D. lablab* display high light efficiency (Figure 2). All of these plants are heliophyte, require full sunlight. Light efficiency depicts fast convert of sun energy. This is in accordance with tendency that herb photosystems are more efficient in converting sun energy than trees (Larcher, 1995) such as existing plants in the Pagak Village, "petai" (*Parkia speciosa*) and "sengon" (*Paraserianthes falcataria*).

### Potency of organic matter as green manure: Energetic and nutritive

Some of the observed crops perceived high calories (energetic) so that if the crops also own high productivity, they will provide more energy to agroecosystem (especially for microbes, soil fauna, herbivores). *P. tetragonolobus*, *M. pruriens utilis* and *Flemingia aspera* represent energetic species (Handayanto *et al.*, 2002). Remnants containing lipid and protein tend to keep higher energy than those containing carbohydrate (Larcher, 1995). Grasses growing in The Brantas River Watershed contain less energy than Dicotyledonae crops (Arisoesilarningsih *et al.*, 2001).

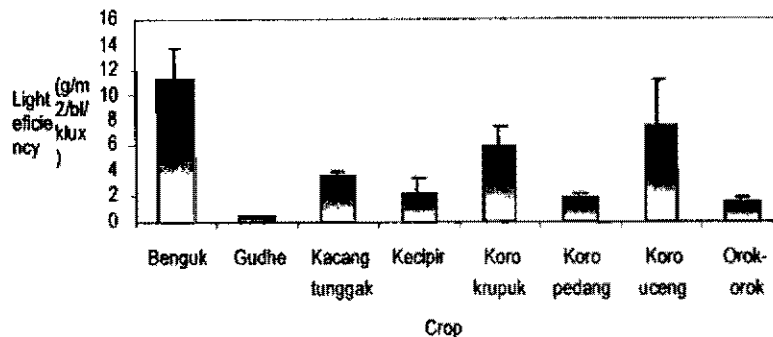


Figure 2. Light efficiency of local flora.

Note: Lines at the bars show relative deviation ( $\alpha = 0,05$ ).

Soil energy concept explains that soil is a unique medium in geo-biosphere, soil obtains energy from geosphere and sunlight. Meanwhile, entire existing systems in biosphere get energy from sunlight (Blum, 1998). To support soil conservation program, soil degradation occurring naturally through pedogenesis or as a result of carbon stock decrease has to be slowed down by giving energy input continuously. Sun energy can protect soil from damages through soil organic matter accumulation (humus) and biological activities following the accumulation. Organic matter will protect soil particles from external force (for example rain and wind) through formation of stable soil aggregates (Blum, 1998). Regrettably that not all of soil surface is covered by vegetation or contains adequate organic matter. Water limitation in dryland causes smaller soil organic matter accumulation; thus, soil degradation due to water erosion happens very easily.

Regardless of lignin and polyphenol contents, except *C. gladiata* and *D. lablab*, all crops yield high quality organic matter because they contain enough C and N so that C/N ratio is smaller than 20. Remnants of some Leguminosae can experience fast or slow decomposition processes depending on their quality. High quality organic matter is shown by high N content and low content of polyphenol and lignin. *Peltophorum dasyrrachis* is grouped into low quality organic matter, while "gamal" (*Gliricidia sepium*) is of high quality (Handayanto *et al.*, 1997).

Some Leguminosae crops contain low P, so that C/P ratio exceeds 200. Only some crops, for example *C. cajan*, *P. lunatus* and *P. vulgaris*, accumulate P in concentration of 0,19 - 0,28 % and C/P ratio ranges between 131 - 182. Even wild plants like bush "paitan" *Tithonia diversifolia* and herb "wedusan" (*Ageratum conyzoides*) contain 0,5 % of P required for fertility of marginal land (Pratikno *et al.*, 2002). These results also indicate that *A. conyzoides* is able to provide P for crop growth at the eight week as many as four times of *T. diversifolia*. Meanwhile, Cairns *et al.* (1998) observed that *T. diversifolia* in Mindanao, the Philippines, accumulates lower P (0,23 % P).

### Monitoring soil biota as affected by multicropping maize

The effect of cover vegetation and root system on soil biological, physical and chemical properties has been investigated periodically. Local crop diversity in farm can improve microbial (Siswanto *et al.*, 2002) and soil fauna (Sari *et al.*, 2002) communities, either as soil decomposers, soil

engineers, or soil transformers. The higher agroecosystem biodiversity is, the more stable food web will be, and the system will also be more tolerance to injuries (weed, pest, disease, harvest failure, etc.). This represents one important characteristic of the sustainable agriculture system.

#### *Decomposition of organic matter of synchronic N or P fertilizer sources*

Synchronization between decomposition of legume remnants and nutrient availability for crop growth is also needed. Remnants of local flora at the limy soil of The Brantas River Watershed, South Malang, cultivated with maize, give significant effects on improving soil mineral N availability. Improvement of soil mineral N is followed by improvement of N absorption by maize. The sequence of maize N absorption is treatment PI> Urea> Mp> Pt > Cp > Fa> Ms> Av > Cm (Sunaryo and Handayanto, 2002).

Pratikno *et al.* (2002) indicate that local flora, especially *A. conyzoides*, can become source of P fertilizer, as also *T. diversifolia*. Efficiency of P composition and P availability at the eight week in *A. conyzoides* is higher (41,44 mg kg<sup>-1</sup> soil) than in *T. diversifolia* (35,03 mg kg<sup>-1</sup> soil).

Based on the above-mentioned description, it is very clear that each crop has its own limitation and excellence. It will be wiser if crops used can be diverse, providing more choices for crop rotation. However, more research on *in situ* exploitation of the crops in multicropping maize is still needed. By accelerating autotrophic succession, the multicropping organic agriculture system is expected to become a healthy and sustainable agroecosystem.

#### Conclusion

The conclusion of this research were: (1) Local flora investigated have varied growth pattern, productivity, water and light efficiencies, nutritive and energetic patterns, depending on species characters; (2) *M. pruriens utilis*, *D. lablab*, *P. lunatus*, *P. tetragonolobus* and *V. sinensis* are drought tolerance and produce high productivity because they posses high light efficiency and high defoliation tolerance; (3) Local crops tolerant to drought at long dry seasons are equipped with deep root architecture, so they remain producing flower and fruit; (4) *C. gladiata*, *P. lunatus* and *M. pruriens utilis* and wild Leguminosae observed have water efficiency of 2,2 - 3,3 g kg<sup>-1</sup> water; (5) Soil covering by diversity of local crops, besides reducing rainwater erosivity, produces crop residues that have potency to function as green manure, improve soil buffering capacities, and increase above- and belowground fauna diversity; (6) Some of cultivated or wild local flora can be exploited as sources of N or P fertilizers that are synchronic with maize requirement.

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## EXPLORATION, DEVELOPMENT AND UTILIZATION OF SOIL MICROBES FOR AGRICULTURE

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### Introduction

Indonesia has a large biological diversity, which is then amplified with the geological diversity. It consists of various organisms from various sources with different habitat. This large diversity is still waiting to be explored, developed, and utilized in order to enrich humanity. Agriculture may take advantage of this large biodiversity. Biochemical processes happening in soil is facilitated by the existence of microbes in the soil. Utilization of soil microbes to improve the process of nutrient transformation, nutrient uptake, toxic substance degradation, and pest antagonism may result in the improvement of crop growth as well as crop productivity.

### Exploration of Soil Microbes

Utilization of soil microbes for agricultural purposes are preceded with the exploration of biological processes occurring in soil that may affect crop productivity. Microbes, which facilitate the biochemical processes, should then be isolated. Selection strategy plays an important role at this stage. Ecological and physiological characteristics of the functioning microbes should be understood in order to construct a working selection strategy.

Understanding the specificity of macro- and microsymbionts in the process of nitrogen fixation, researchers in our laboratory are utilizing soybean to isolate *Rhizobium japonicum*, the microsymbiont in the nitrogen fixation process of soybean nodules (Jutono, 1984; Aristyaningsih, 1994). Using maize as the macrosymbiont, we have obtained several isolates of vesicular arbuscular mycorrhizal fungi that belong to the genus of *Glomus* (Kabirun and Baon, 1982 and Haryuni, 2002).

Selective media could also be used to isolate specific microbes. Several isolates of *Azotobacter* sp., a non-symbiotic nitrogen-fixing bacterium, have been isolated using nitrogen free medium (Soesanto and Wedhastri, 1983). Using Pikovskaya media containing insoluble phosphate as their sole phosphorus source, Widada *et al.* (1995) and Katmanto (1997) isolated several phosphate solubilizing bacteria from vertisol, oxisol and ultisol, soils having problem with phosphorus availability. The bacteria belong to the genus of *Pseudomonas*. Prijambada *et al.* (in preparation) have isolated *Acinetobacter* sp. from peat soil of Rawa Pening, Central Java, by the same method. Rhizobacteria having an ability to produce plant growth promoting substances have been isolated using medium that has a high osmotic pressure (Ngadiman *et al.*, 2000).

Isolation of microbes may be facilitated by special environmental conditions that work as a natural selection pressure. Several isolates of fungi having capability to oxidize elemental sulfur, which belong to the species of *Aspergillus japonicum*, *A. flavus* and *Penicillium naviogenes*, have been isolated from soil near the crater of Mt. Dieng in Central Java (Sumarsih, 2001). An isolate of *Streptomyces violaceusniger*, which has a capability to antagonize the growth of *Streptomyces scabies*, was isolated from potato tuber periderm (Ngadiman *et al.*, 1993). Several isolates of *Streptomyces*, which have capabilities to hydrolyze chitin, were isolated from a condiment made from fermented shrimp (Khadarwati, 2001).

Following the postulate of Robert Koch, the obtained isolates should be re-examined for their ability to perform soil biochemical processes and compared to their alternative processes. Jutono (1969a), Hartini (1987) and Suryanti (1995) show the role of *Rhizobium* sp. in supporting the growth of leguminous crops. Sulistiyanto (1998) examined the role of vesicular-arbuscular mycorrhiza in the

nutrient uptake and growth of maize. Kabirun and Widada (1995) also examined the role of vesicular-arbuscular mycorrhiza in the growth of soybean. Astuti (1998) shows the role of phosphate solubilizing bacteria in the absorption of phosphorus as well as the growth of maize in vertisol and ultisol, while Widada *et al.* (2002) show the role of double inoculation between phosphate solubilizing bacteria and arbuscular mycorrhiza in the growth of sorghum. Ngadiman *et al.* (1993) shows the role of the isolated *Streptomyces violaceusniger* in preventing potato scab caused by *Streptomyces scabies*. Indarti *et al.* (2001) show the work of the isolated chitin degrading *Streptomyces* in suppressing the affliction of *Pratylenchus coffeae* in coffee.

Our study on the mechanism of phosphate solubilization revealed that the ability of microbes to solubilize calcium phosphate is related to their ability to produce gluconic acid, while the ability to solubilize aluminum and iron phosphate is related to the ability to produce citric acid (Priambada *et al.*, in preparation). Based on those results we isolated several fungi, which belong to the genus of *Aspergillus* and *Penicillium* and have an ability to produce and secrete citric acid.

### Preparation of Microbial Inoculants

To bring the benefit of biochemical processes facilitated by the isolated microbes into field, microbial inoculants should be prepared. For a microbial inoculant to be commercially successful, it must be economically mass-produced and then formulated into a form that is cost-effective and readily applicable by the end user. Mass production of microbes can be done by submerged cultivation or solid state cultivation (Walter and Paau, 1993).

In a submerged cultivation, a monoculture of a microorganism is aseptically inoculated into a sterile medium containing nutrients (Walter and Paau, 1993). The medium is agitated to provide homogenous environment, and O<sub>2</sub> or air is often sparged into the medium to supply O<sub>2</sub> to aerobic organism. A batch-submerged cultivation possesses a problem of inefficient carbon consumption and a long lag phase. Jutono (1969) innovated a biphasic system, which is formerly used for antibiotic production, to obtain a high amount of biomass. As biomass productions with big fermentor often face a risk of contamination, biphasic cultivation in a large number of small flasks rather than big fermentor is then used in our laboratory to produce *Rhizobium* biomass. Wedhastri (2001) found that mannitol is essential for growing *Azotobacter chroococcum*.

Solid-state cultivation is the growth of organisms on a solid substrate in the absence of any free water (Walter and Paau, 1993). The solid-state cultivation has the advantage of low medium cost and low capital investment. For biomass production of his *Streptomyces violaceusniger*, Ngadiman *et al.* (1993) used solid-state cultivation. Hartono (2003) and Sastro (2001) also used the same method to produce chitin degrading Actinomycetes and spores of rock phosphate solubilizing *A. niger*, respectively. In order to minimize their production cost, economically acceptable-yet easily available materials are used for cultivation media. Ngadiman *et al.* (1993) used a mixture of wheat brand, swine manure, and Ca(OH)<sub>2</sub> as cultivation media, Sastro (2001) used a mixture of rice brand, starch and tapioca waste, while Hartono (2003) used a mixture of rice brand, starch and poultry manure for that purpose.

Practicality of the biomass to be used as inoculants is dependent on their formulation. Viability of the microbes in the inoculants is also dependent on the formulation. Vincent (1970) stated that viability of microbes in liquid microbial inoculants is very low. For that reason, we decided to formulate our microbial inoculants in a solid form. Using a mixture of peat and clay as a carrier in the inoculants, Jutono (1969b) show that after 9 months storage, the number of viable *Rhizobium* in the inoculants still reaches  $5.7 \times 10^6$  when stored at 4°C. However, the number could drop to only  $3.2 \times 10^3$  when stored at 30°C. A mixture of peat and zeolit was also found to support the shelf life of *Azotobacter chroococcum* (Wedhastri, 2002). Sastro (2001) shows a high viability of *Aspergillus niger* spores that are mixed with rock phosphate.



### Examination of Inoculant Effectiveness

The success of soil biochemical process improvement, which is facilitated by microbes in the inoculants, should be examined in the soil microcosm as well as in the field. Primarily, it is dependent on the ability of the microbes to survive their environment selection. Jutono (1969a) examined the effectiveness of *Rhizobium* inoculants in supporting the growth of soybean in Tropopsament. It increases nodulation, number of seed per 100 plants, dry weight of seed per 100 plants, all by 50%. We are now working on the ability of phosphate solubilizing *A. niger* to colonize rhizosphere and support the growth of maize grown in soils with problem in phosphorus availability. We are also working on the same subject for chitin degrading *Actinomycetes*.

### Conclusion

Laboratory of Soil and Environmental Microbiology, Faculty of Agriculture, Gadjah Mada University has been working extensively in the exploration, development, and utilization of soil microbes for agriculture. We are fully aware that direct selection of soil microbes in selective media could decrease the diversity of microbes that undergo selection. Widada (in preparation) found that direct selection on agar plate could reduce the variety of microbes that are isolated. In-situ and ex-situ enrichment using selective nutrition could increase the diversity of microbes that are going to be selected.

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CONSERVATION AND SUSTAINABLE MANAGEMENT  
OF BELOWGROUND BIODIVERSITY IN INDONESIA  
IN THE CONTEXT OF THE GLOBAL CSM-BGBD PROJECT

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## Introduction

Poverty, associated with increasing pressure on the land in the humid tropics, is a major driver of global change, leading to accelerated habitat modification and fragmentation of remaining pristine areas. Although it is not apparent to the naked eye, soil is one of the most complex habitats on earth and contains one of the most diverse assemblages of living organisms. As part of a move to make up for the neglect of this aspect of biodiversity in much of the current discussion and policy frameworks for biodiversity, the CSM-BGBD project has been initiated to assess impacts of land use change on belowground biodiversity. The project aims to clarify the function of soil biota along gradients of agricultural intensification around global biosphere forest reserves in Brazil, Côte d'Ivoire, India, Indonesia, Kenya, Mexico, Uganda. Our work will include i) making a comprehensive inventory of soil biota and ii) developing a strong theoretical framework linking the documented belowground biodiversity to demonstrated soil functions. In this overview of the how the global project connects to the specific efforts in Indonesia, we will consider (on the basis of the various international workshops and consultations):

- The conceptual framework,
- The global sampling design,
- An operational definition of 'intensification' at plot and landscape scale,
- The sampling scheme for Indonesia,
- From data on soil biota to 'sustainable management' of belowground biodiversity,
- Organizational matters.

## The Conceptual Framework

Few people will doubt that the biodiversity value of any piece of land will decrease with increasing intensity of agricultural management, aimed at harvesting crops that may or may not be part of the original flora of the area, but have undergone 'domestication' that makes them less compatible with other parts of the local ecosystem (which become labeled as 'pest and diseases'). The shape of the 'trade-off' curve between biodiversity value and land use intensity is, however, less certain. Yet, this shape has major implications on how societies can best achieve a balance between biodiversity conservation and production of food, feed and fiber.

Three hypotheses indicated in Figure 1 are:

**Hypothesis 1:** *'The most valuable components of the local flora and fauna are likely to be sensitive to and incompatible with agricultural use, and will thus tend to disappear (or be eradicated) in early phases of land use intensification; once this part of the biodiversity is lost further intensification will be of little consequence for on-site loss of biodiversity, but may actually help in as far as higher yields per unit area decrease 'land hunger' for further agricultural expansion'*

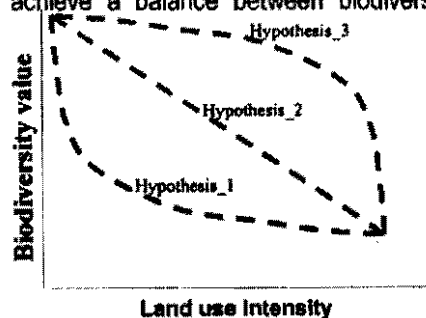


Figure 1. Three hypotheses on the way 'biodiversity value' will tend to decrease with increasing 'land use intensity'; for further discussion see text.

**Hypothesis 2:** 'Loss of biodiversity value is approximately proportional to the increase in land use intensity'

**Hypothesis 3:** 'by appropriate management of above- and below-ground biota, optimal conservation of biodiversity for national and global benefits can be achieved in mosaics of land-uses at differing intensities of management and furthermore result in simultaneous gains in sustainable agricultural production'

The CSM-BGBD project wants to test this third, 'optimistic' hypothesis. Yet, empirical evidence is thin on the ground, so outcomes closer to hypothesis 2 or even 1 would not be unexpected. Evidence for plant species richness obtained during the surveys of the Alternatives to Slash and Burn (ASB) project in Jambi (Figure 2) indicate an outcome that may be close to hypothesis 2, although in more limited parts of the range both the convex curve of hypothesis 1 and the concave curve of hypothesis 3 can be recognized (if one wants...).

If we compare such data with the hypotheses, however, we can see a number of important challenges to empirical work, referring to the X and the Y-axes of the graph:

- a) The hypotheses (and Figure 1) speak of biodiversity 'value', while the survey data are primarily expressed in numbers of species observed; there are many steps to be taken between the primary observations by soil biologists and the 'value' that different stakeholders in society assign to it,
- b) The concept of 'agricultural intensification' is used loosely in a ranking of different agricultural systems, but the shape of the curves (convex or concave) can change according to the way 'land use intensity' is defined quantitatively.

In the CSM-BGBD project, we aim to address both of these challenges, by going beyond the 'survey' stage into an exploration of the functional value of soil biota for farmers and external stakeholders, and by using an operational version of the 'land use intensity' concept.

The data in Figure 2 refer to plant species richness in plots of standard size. Plants live both above and belowground, so plant species richness can be a first indicator of both parts of the ecosystem. However, aboveground parts of plants provide both the structure and primary production of food sources for all other parts of the ecosystem, and may thus be used as first indicator. Belowground, however, plant roots are only one of the contributing elements to the structure of the ecosystem, and provide only part of the energy basis for the food web. It is likely that changes in above- and belowground biodiversity can at least be partially uncoupled (Figure 3). Again, there is little consistent data on this topic, so the data collection of the CSM-BGBD project may become a benchmark in the discussion on the topic (Bignell *et al.*, in press; Gillison *et al.*, in press). If the relation is

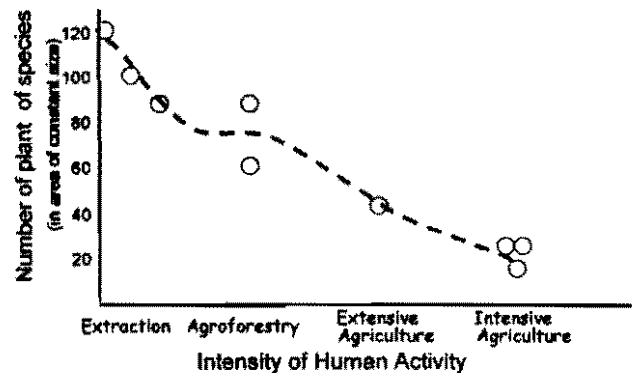


Figure 2. Plant species richness across a land use intensity gradient in Jambi (Murdiyarsa *et al.*, 2002; Van Schaik and Van Noordwijk, 2002).

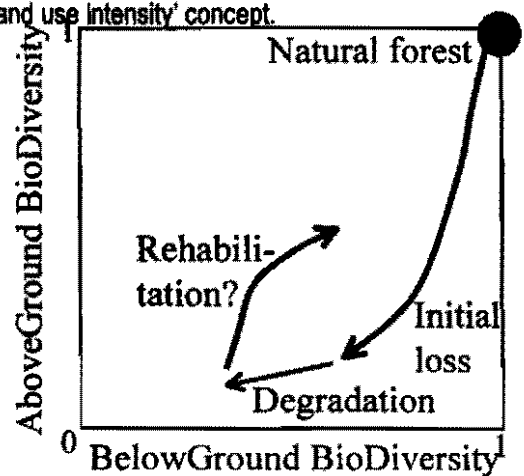


Figure 3. The dynamics of above- and belowground biodiversity may be partially uncoupled, as aboveground losses can be more rapid, but may also be more easily reversible

as depicted in Figure 3, we may tentatively conclude that hypothesis 3 holds for BGBD even in situations where aboveground biodiversity changes according to hypothesis 2.

The starting point for the BGBD project will thus be to establish, at the plot and landscape level, the connections between aboveground and belowground biodiversities and between either of these and land use intensity.

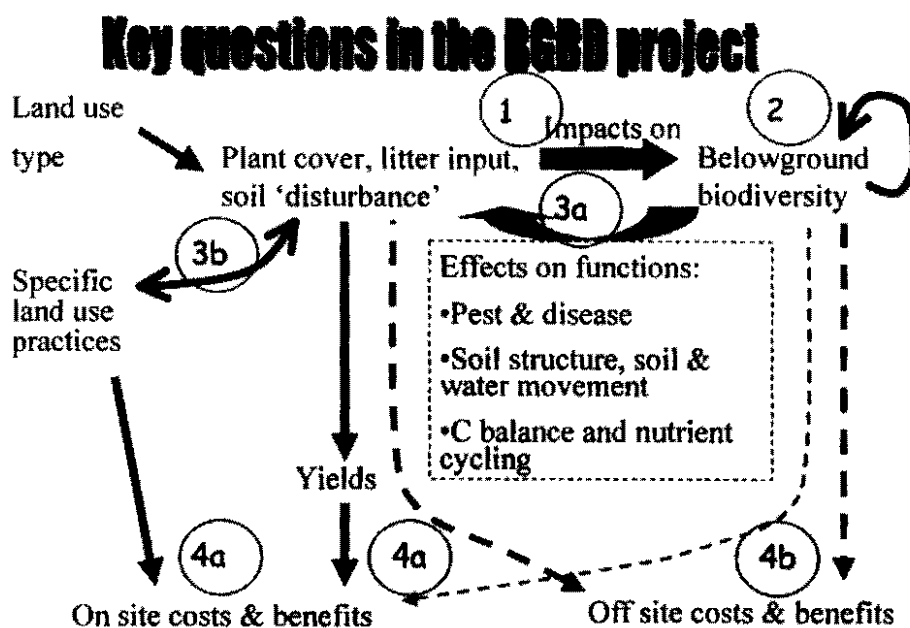


Figure 4. Diagram of the four main questions for the CSM-BGBD project.

As summarized in Fig 4, we will analyze the relationship between land use practices and belowground biodiversity in a number of steps or questions:

- How do land use change and specific management practices within broad land-use categories impact on soil biota?
- How do the various soil biota function in belowground food webs in different ecosystems?
- 3a. What are the key functional roles of soil biota in agro ecosystems? And, which groups play these roles?
- 3b. How can farmers, as managers of agro-ecosystems, work through (or with), rather than against soil biota?
4. How do the presence of specific soil biota, and the diversity of the belowground ecosystem as such, contribute to the overall cost – benefit balance at the farm, landscape and global levels?

A first analysis of the food web structure for the data collected during the ASB surveys, suggests that the occurrence of the 'top predator' group among the soil invertebrate fauna is closely linked to the total flow of organic inputs (from litter fall, root turnover and remnants of previous vegetation) in these systems. This may suggest a 'hypothesis 2' type relationship, but data for Jambi and Lampung show some differences that need to be further explored.

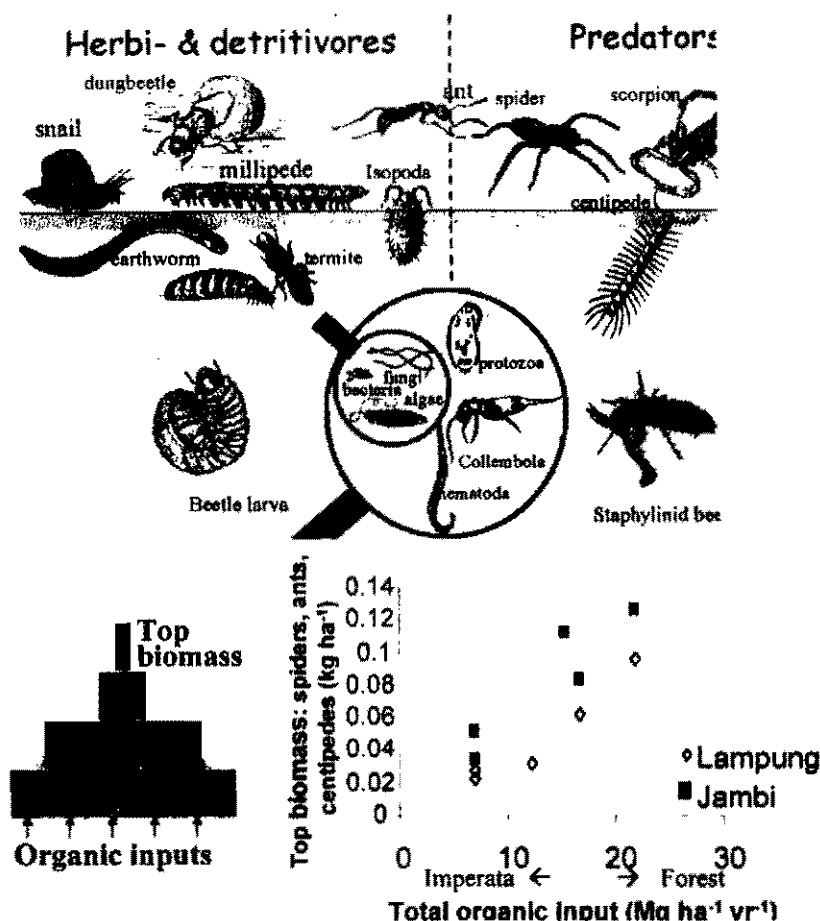


Figure 5. Key groups of soil biota that can be ranked into a food web or ecological pyramid; data for the ASB surveys suggest a general relationship between total organic inputs and the biomass of spiders plus centipedes plus ants based on (Hairiah *et al.*, 2001; Susilo *et al.*, *in press*).

### The Global Sampling Design

The research design for the global survey of belowground biodiversity in relation to intensification of land use is based on a number of layers:

- Countries representing the full spectrum of population densities and land use intensities within the (sub) humid tropics,
- Benchmarks within those countries that add to the spectrum at country level,
- Windows or landscapes that are selected to represent the full spectrum of land use intensities within the benchmark,
- Points representing the full spectrum of land use intensities within the window.

While the seven countries that participate in the CSM-BGBD project cannot fully represent the full spectrum of situations within the three tropical continents, they do represent the full spectrum of national-scale population densities in what are recognized as global 'hot spots' of biodiversity.

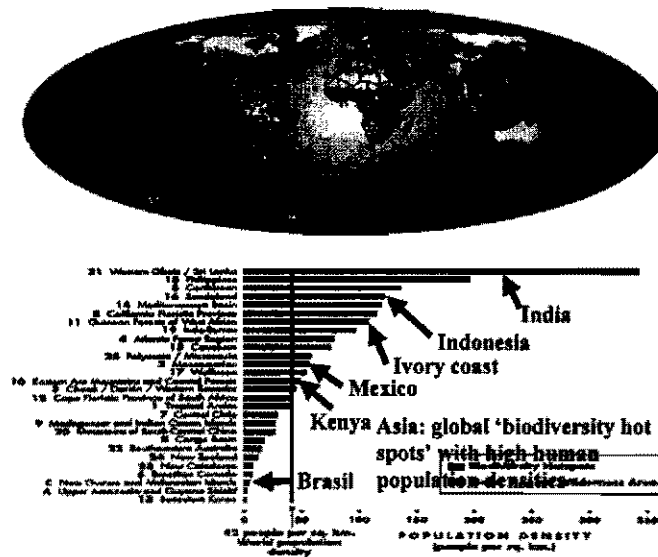


Figure 6. Global hot spots of biodiversity and the national-scale human population densities (quoted in Williams *et al.*, 2001).

In each of these 7 countries we expect to sample in four types of land use (forest, tree-crop production systems, annual-food crop systems and pasture), with a range of intensities in the three first groups, making 7 land use types in each country. Of course, the specific practices and crops in these systems will differ between countries and benchmarks, but if we want to test hypotheses at the level of 'land use intensity' we should not shy away from including jungle rubber and multistrata coffee into the 'extensive tree crop' class, and 'monoculture plantation rubber and sun coffee in the intensive class.

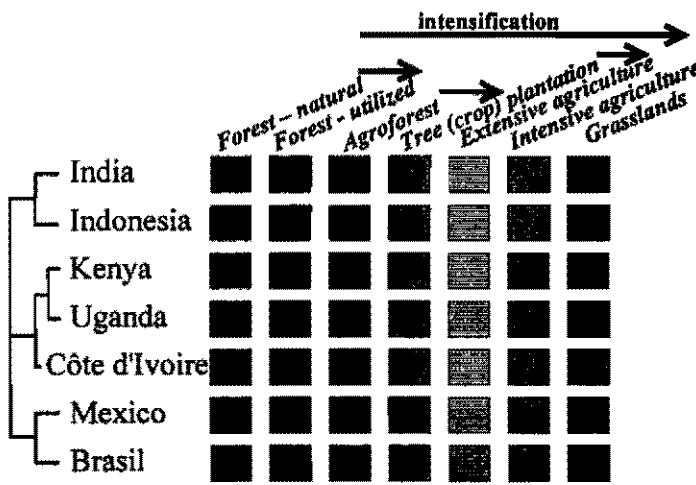


Figure 7. Global sample design, aiming for representative of 7 land use classes in each of 7 countries, to test both hypotheses on 'within land use class' intensification, and those that compare across the full spectrum.

The basic research design, and the levels of data that need to be represented in the global database, is summarized in Figure 8.

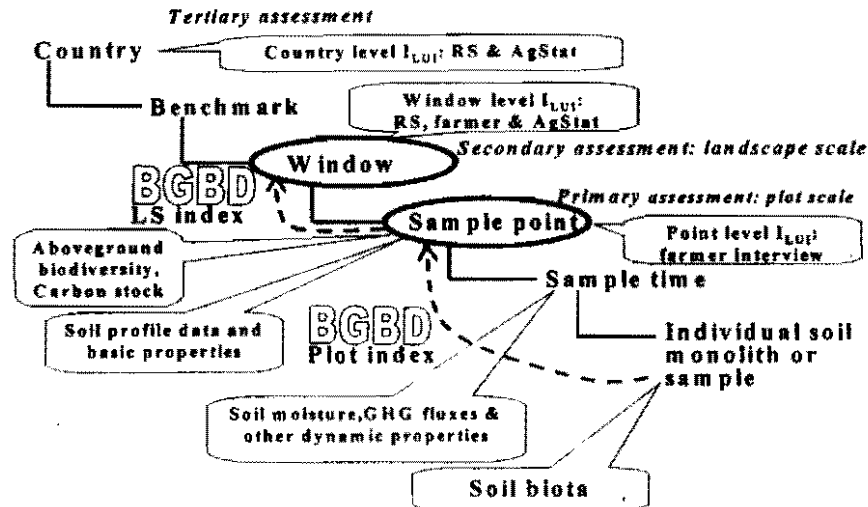


Figure 8. Overall research design, with a focus on data analysis at the 'sample point' and 'landscape' level, where underlying data on soil biota are compiled into a BGBD-index that can be compared with a land use intensity index.

### An Operational Definition Of 'Intensification' At Plot And Landscape Scale

The central CSM-BGBD hypothesis refers to 'intensification' of agriculture. Other 'Integrated Natural Resource Management' research is equally interested in the balance between productive and environmental functions in agriculturally used landscapes. To test such hypotheses we need to be clear on how to measure 'intensity' of land use systems. While in most case studies the term intensification is used in a relative sense, referring to specific practices, a more generic concept is usually implied.

In agricultural economics, the term 'intensity' is used for 'total production factor input' per unit of land, with monetary equivalents for factors such as labour, fertilizer, pesticides, growth regulators and agricultural machinery. From the perspective of the use of a limited amount of financial resources, 'intensification' is juxtaposed to 'extensification' (as acquiring additional land competes with the capital needs for obtaining inputs), but at the scale of a larger scale strategy both may be needed to meet the expected future demand. Ideally, economic valuation of the various inputs used should take into account the environmental costs associated with them, but currently such costs often remain external to the price-forming mechanisms and considerations. For discussions of 'integrated natural resource management' consequences of intensification we may thus need other 'currencies' to estimate the combined and interactive effect of various measures to increase agricultural production.

A first attempt at a generic land use intensity Index was formulated by Giller *et al.* (1997), combining the Ruthenberg (1980) cropping index, with terms for the use of fertilizer, pesticides, irrigation and mechanization. The index, however, needs to be further operationalized, and may have to include other dimensions of intensification. Our '*Intensification*' concept has to cover the full range from very extensive 'shifting cultivation' systems to intensive horticulture, where the chemical, physical as well as biological properties of the rooting medium are under complete technical control. It has to respond to increases in the fraction of time land is used for crop production, the fraction of total biomass harvested, the amounts of fertilizer, irrigation and pesticides used, as well as the amount of fossil energy used in soil



tillage and mechanized farm operations (including harvest). The resulting index should preferably be dimensionless, so we have to choose appropriate 'scaling factors' for all the elements of the equation.

A first step in developing the index is to recognize five parts of an agriculturally used landscape:

$$R_{rot} + R_{per} = 1 \tag{1a}$$

where

$R_{rot}$  = fraction of the area used for rotational systems,

$R_{per}$  = fraction of the area used for permanent systems, with no 'open field' stage

$$R_{rot} = R_{crop} + R_{fallow} \tag{1b}$$

$R_{crop}$  = fraction of the total area used for annual or tree crops as part of a rotational system,

$R_{fallow}$  = fraction of the total area left as fallow part of a rotational system, with some potential use for grazing and production of firewood and NTFP's,

$$R_{per} = R_{pas} + R_{for} + R_{ref} \tag{1c}$$

$R_{pas}$  = fraction of the area used for permanent pasture or grazing land

$R_{for}$  = fraction of the total area used for permanent 'forest' or 'tree based' systems (with internal regeneration but no 'open field' phases) (please note that classical plantation forestry belongs to the 'rotational' category, while selective logging or agroforests with forms of 'sisipan' rejuvenation belong here),

$R_{ref}$  = fraction of the total area left for 'refugia and filters' (landscape elements that consist largely of 'border' and for which 'area' is not a straightforward concept).

Intensification can be based on a reduction of the  $R_{per}$  and  $R_{fallow}$  fractions of the land use, as well as an increase of intensity within the  $R_{crop}$  or  $R_{pas}$  fractions. We here assume that the 'refugia and filter' fractions have no or negligible direct production functions (otherwise this land is supposed to belong to one of the two other fractions).

In the shifting cultivation -> long fallow -> short fallow -> permanent cropping range, we can make use of Ruthenberg's *cropping index* or index of land use intensity. In a 'steady state' form we can equate the time fractions to area fractions. The Ruthenberg index then reads  $T_{crop} / (T_{crop} + T_{fallow})$ , where  $T_{crop}$  is the length of time (or the fraction of area) cropped, and  $T_{fallow}$  is the length of time (or the fraction of area) under a fallow of zero use intensity. Where the fallow vegetation is also used for harvestable products (e.g. through grazing or production of firewood), we may want to include it in our intensity concept on the basis of the 'harvest index', the fraction of total biomass harvested (either as consumable product, as crop residues used for fodder, or removed through the use of fire). This same '*off take index*' (a term broader than the '*harvest index*' as used in agronomic studies) may well be used in the 'cropping phase' to distinguish between situations where only grain (or tuber) is harvested and those where all crop residues are removed from the field as fodder. For the cropping phase we include *fertilization* (relative to nutrient removal at crop harvest), *irrigation* (relative to total water use by the crop), soil tillage and mechanization (based on the *fossil energy used* per ha relative to the energy content of the crop harvested) and the use of *pesticides* (based on 'active ingredients' and their half-life time). These intensity factors can apply both to an 'annual crop dominated' and a 'tree dominated' of fallow stage of a cyclical production system.

Combining these elements, we get the following equation for a 'land use intensity' index ( $I_{LUI}$ ):

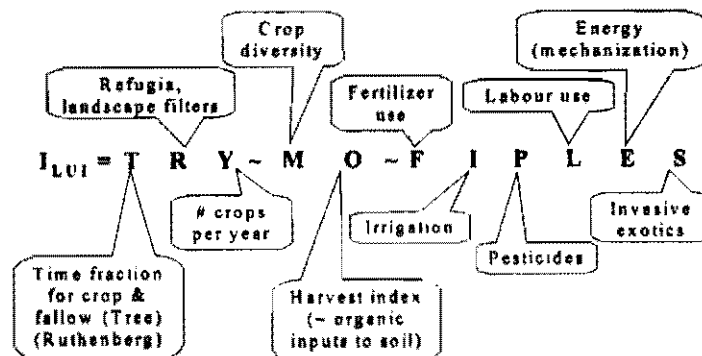


Table 1. Parameters of the index of land use intensity, and their representation at landscape scale

Aspects of land use intensification that may impact on belowground biodiversity	Factor in plot-level 'LU Intensity index'	'Macro' indicators
Increasing time-fraction of annual food crops, leading to increase of 'open time' and reduced organic input	T	Remote sensing, agricultural production statistics
Reduction in landscape fraction of filters and refugia	R	Remote sensing
Yearly number of crops and concomitant increase in weeding intensity – reducing litter input to the soil, and increasing 'open time' of the soil at early stages of any new crop	Y	--
Reducing diversity of annual crops	M <sub>c</sub>	Market, agricultural production statistics
Replacing mixed woody fallow by planted tree monoculture	M <sub>r</sub>	Remote sensing, agricultural production statistics
Increasing harvest fraction of aboveground biomass	O <sub>h</sub>	Animal production statistics, type of fuel used
Increasing burn fraction of aboveground biomass	O <sub>b</sub>	Smoke/haze production
Inorganic fertilizer	F	Trade statistics for fertilizer
N		
P		
Lime		
Inundation/irrigation (e.g. paddy rice)	I	Remote sensing, Agricultural statistics
Pesticide use	P	Trade statistics for pesticides
Fungicides		
Insecticides		
Herbicides		
Other		
Trampling and soil compaction by	L	--
People		
Animals		
Tractors		
Soil tillage	E	Agricultural statistics
Manual		
Animal traction		
Tractors		
Introduction and spread of invasive exotics	S	--
Lack of understanding of role of soil biota	--	Knowledge-survey

A more specific equation can be:

$$I_{LUI} = \left( \begin{aligned} & \left( \frac{R_c t_c}{t_c + t_f} \right) (y_c) \left( 1 + \frac{1}{M_c} \right) \left( \frac{B_{h,c} + B_{f,c}}{B_c} \right) \left( 1 + \frac{N_{fertilized,c}}{n_c B_c} \right) \\ & \left( 1 + \frac{W_{irrigated,c}}{10 B_c / w_c} \right) \left( 1 + \sum_i \frac{P_{used,c,i} T_{1/2,i}}{p_i} \right) \frac{L_c}{100} \left( 1 + \frac{E_{utilized,c}}{e_c B_c} \right) \\ & + \\ & \left( \frac{R_f t_f}{t_c + t_f} + R_p \right) \left( 1 + \frac{1}{M_f} \right) \left( \frac{B_{h,f} + B_{f,f}}{B_f} \right) \left( 1 + \frac{N_{fertilized,f}}{n_f B_f} \right) \\ & \left( 1 + \frac{W_{irrigated,f}}{10 B_f / w_f} \right) \left( 1 + \sum_i \frac{P_{used,f,i} T_{1/2,i}}{p_i} \right) \frac{L_f}{100} \left( 1 + \frac{E_{utilized,f}}{e_f B_f} \right) \end{aligned} \right) (1 - R_{ref})^r S$$

Where the subscripts c and f refer to a crop (annual crops) and fallow (or perennial crops) phase of the land use system, and

$t_c$  and  $t_f$  = length of cropping or fallow period in a typical rotation [year],

$y_c$  = yearly number of cropping seasons,

$M_c$  and  $M_f$  = number of crops planted per cropping season in the same field or number of trees planted,

$B_c$  and  $B_f$  = (final) total biomass of a crop or fallow vegetation [ $Mg\ ha^{-1}$ ],

$B_{h,c}$  = (cumulatively) harvested part of the biomass of a crop or fallow vegetation [ $Mg\ ha^{-1}$ ],

$B_f$  = biomass burnt [ $Mg\ ha^{-1}$ ],

$N_{fertilized}$  = the amount of plant nutrients (N + P + K) added to the field as external fertilizer (in inorganic or organic form, the key is that it is derived from outside of the 'system' under consideration) [ $kg\ ha^{-1}$ ],

$n_c$  = typical nutrient (N + P + K) concentration [ $kg\ Mg^{-1}$ ],

$W_{irrigated}$  = amount of water provided by irrigation during one cropping year [mm],

$w$  = water use efficiency of the crop, or biomass production per unit of water transpired [ $kg / l$ ] (the factor 10 is required to make the term dimensionless),

$E_{utilized}$  = sum of fossil energy used for all soil tillage and mechanized harvest operations [ $MJ\ ha^{-1}$ ],

$e$  = typical energy content of crop biomass [ $MJ\ Mg^{-1}$ ],

$P_{used}$  = total amount of active ingredient of pesticides used [ $kg\ ha^{-1}$ ],

$T_{1/2}$  = half-life time of the active ingredient [year],

$p$  = a biological impact rating of the various active ingredients [ $kg\ year\ ha^{-1}$ ],

$R_{ref}$  = landscape fraction left for refugia and filters [],

$r$  = power of the refugia factor [],

$S$  = multiplier for the (irreversible) spread of invasive exotics.

Some examples for increasing intensity within the  $R_{rot}$  domain are:

System	$I_{LUI}$	Total yield Mg ha <sup>-1</sup> yr <sup>-1</sup>	$t_c$ yr	$t_f$ yr
Shifting Cultivation - no inputs, no harvest from fallow	0.03	0.13	2	30
Shifting Cultivation - no inputs, some harvest from fallow	0.03	0.31	2	30
Shifting Cultivation - no inputs, fallow products harvested	0.03	1.06	2	30
Long Fallow - no inputs, no harvest from fallow	0.07	0.33	2	10
Long Fallow - no inputs, some harvest from fallow	0.07	0.50	2	10
Long Fallow - no inputs, fallow products harvested	0.08	1.17	2	10
Short Fallow - no inputs, no harvest from fallow	0.11	0.46	2	5
Short Fallow - no inputs, some harvest from fallow	0.12	0.60	2	5
Short Fallow - no inputs, fallow products harvested	0.14	1.17	2	5
Permanent cropping - no inputs	0.40	1.20	4	0
Permanent cropping - low fertilizer rate	0.48	1.60	4	0
Permanent cropping - idem, higher harvest index	0.73	2.40	4	0
Permanent cropping - idem, higher fertilizer rate	0.85	3.60	4	0
Permanent cropping - idem, pesticide use	1.47	4.20	4	0
Permanent cropping - idem, fully mechanized	2.09	4.20	4	0
Permanent cropping - idem, double cropping + irrigation	4.00	6.00	4	0

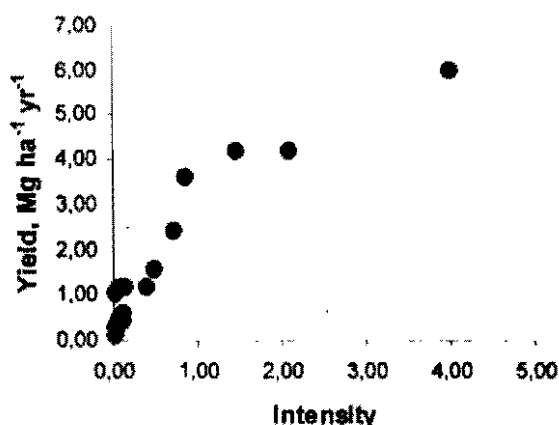


Figure 9. Relationship between total harvested yield and 'intensity' index LUI for a number of cropping systems.

In this equation variations in the amount of biomass harvested in 'no input' systems can lead to the intensity indices of 0 - 1, while the use of fertilizer will double the result for nutrient application rates up to 'balanced nutrient budget' level and more for higher rates, and the use of pesticides, fossil energy and irrigation can lead to higher values. The index is 'open ended' on the right hand side, and values above 10 are possible.

To utilize this index for a particular cropping system, we will thus need to collect data on the typical duration of crop and fallow, the amounts of biomass harvested from both phases of the cycle, the amounts of fertilizer (N + P + K expressed as nutrient application rates) and irrigation water. The total use of fossil energy may be derived from fuel use for tractors and the like. Pesticides should be recorded as

amounts of active ingredient, and we will need to get some expert advice on half life times and the index of overall biological impact of the active ingredient.

A number of the further conversion factors needed, such as typical nutrient content of harvested products and water use efficiency of the crop may be derived from existing databases.

If indeed we want to use this index, we have to make sure we have protocols (questionnaire) to measure, assess or estimate

$t$  = length of cropping or fallow period in a typical rotation [year] (interview the farmer for plot history)

$B$  = (final) total biomass of a crop or fallow vegetation [ $\text{Mg ha}^{-1}$ ] (destructive biomass sample, use of allometrics for woody perennials)

$B_h$  = (cumulatively) harvested part of the biomass of a crop or fallow vegetation [ $\text{Mg ha}^{-1}$ ] (interview the farmer for plot history)

$N_{\text{fertilized}}$  = the amount of plant nutrients (N + P + K) added to the field as external fertilizer (in inorganic or organic form, the key is that it is derived from outside of the 'system' under consideration) [ $\text{kg ha}^{-1}$ ] (interview the farmer for plot history)

$n_c$  = typical nutrient (N + P + K) concentration [ $\text{kg Mg}^{-1}$ ] (for crops there are databases that we can use, occasionally we may need to sample ourselves)

$W_{\text{irrigated}}$  = amount of water provided by irrigation during one cropping year [mm] (interview the farmer for plot history)

$w$  = water use efficiency of the crop, or biomass production per unit of water transpired [ $\text{kg l}^{-1}$ ] (the factor 10 is required to make the term dimensionless) (database to construct lookup table for local climate and  $C_3$  versus  $C_4$ )

$E_{\text{utilized}}$  = sum of fossil energy used for all soil tillage and mechanized harvest operations [ $\text{MJ ha}^{-1}$ ] (interview the farmer for plot history)

$e$  = typical energy content of crop biomass [ $\text{MJ Mg}^{-1}$ ] (database for look-up-table based on harvested product)

$P_{\text{used}}$  = total amount of active ingredient of pesticides used [ $\text{kg ha}^{-1}$ ] (interview the farmer for plot history)

$T_{1/2}$  = half-life time of the active ingredient [year] (database)

$p$  = a biological impact rating of the various active ingredients [ $\text{kg year ha}^{-1}$ ] ('expert' rankings?)

### The Sampling Scheme For Indonesia

The sample design for Indonesia was discussed at a number of global workshops, to ensure that it will fit well within the overall data set, and can contribute to the global evaluation of our key hypothesis.

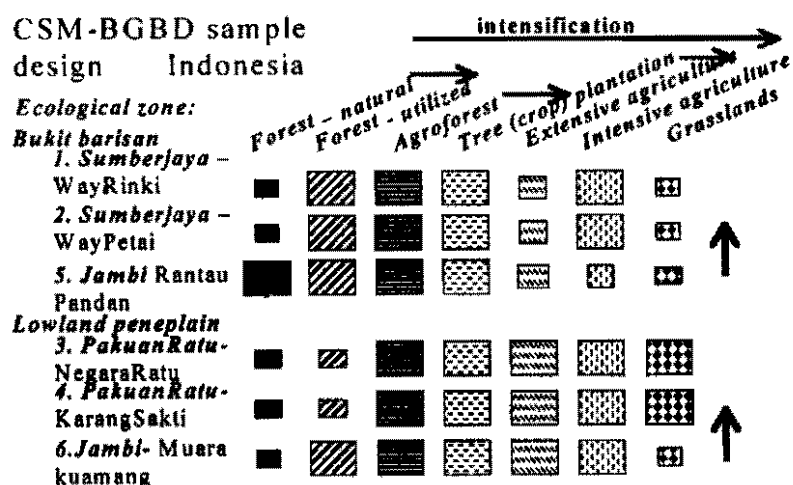


Figure 10. Expected presence of the 7 land use types across the 6 sampling windows for Indonesia.

Table 2. Indonesia's sample design

Aspects of land use intensification	Level in the sample design
Tropical countries: Brasil -> India	7 countries (5 – 350 persons km <sup>2</sup> )
Benchmarks (windows) within country: e.g. Jambi versus Lampung	3 benchmarks (range of 'forest cover'), 2 windows each
Forest – grasslands continuum	4 main land use types
Intensity of use within the 'forest', 'tree crops' and 'food crop' land use classes	2 levels in 3 land use types (all together 7 land use types)
Sample-point level 'Index of land use intensity' characterized by $I_{LUI}$	3 replicates per land use type per window: in Indonesia: $6 * 7 * 3 = 126$ sample points, sampled on two occasions (wet, dry)

Not all windows may contain good representation of all 7 land use types, but across the 6 windows we should be able to obtain a fair spectrum of situations.

The selection of sample points within the windows will be essentially based on a grid sampling, but on the basis of an *a priori* classification of land use, we will add points to ensure all land classes are represented, and we will randomly select among points that appear to be similar in the dominant land use categories. The final result will be similar to a 'stratified random' sampling scheme, but differ in maintaining a minimum distance between sample points (thus ensuring 'independence' from the perspective of the dispersal distance of most soil biota).

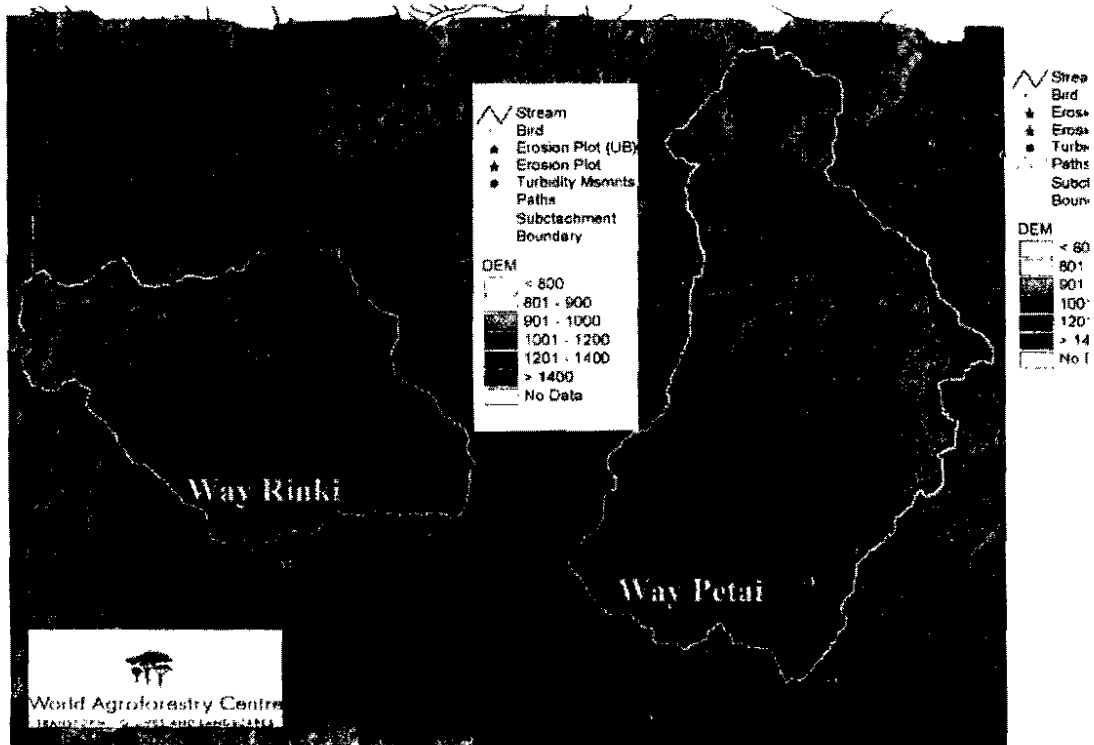


Figure 12. Two 'windows' will be sampled in the Sumbarjaya benchmark, both of which are a 'subcatchment' of about 20 km<sup>2</sup> that include a part of the remaining forest on Bukit rigis (with a gradient of human use intensity starting from the edge of the forest), a range of coffee gardens ('sun coffee' and 'shade coffee), intensive crop production (horticulture) and some temporarily abandoned land (*Imperata* grassland). These windows are also instrumented for hydrological research by another project.

In the mean time, household and community level survey and discussions can help us understand *who* is applying the various 'threat factors' on their farms, and *why* they do this. This may help to recognize the various trade-offs involved.

Subsequently a more direct empirical approach can quantify the role of the various threats (e.g. effects of pesticide use or reduction of surface mulch), and discussions with farmers can lead to identification of possible entry points for more biodiversity-friendly farm management practices.

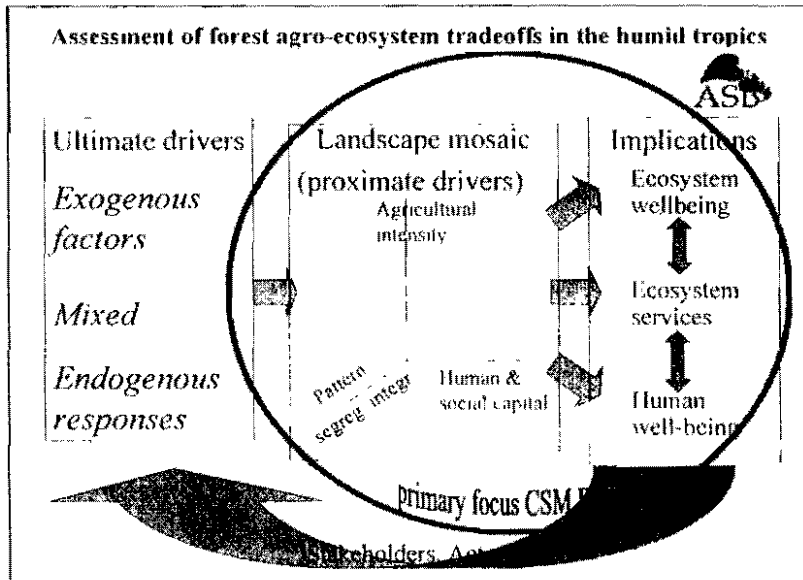


Figure 14. Components of the wider 'land use system' that are assessed in the current Millenium Ecosystem Assessment on the forest-agriculture interface.

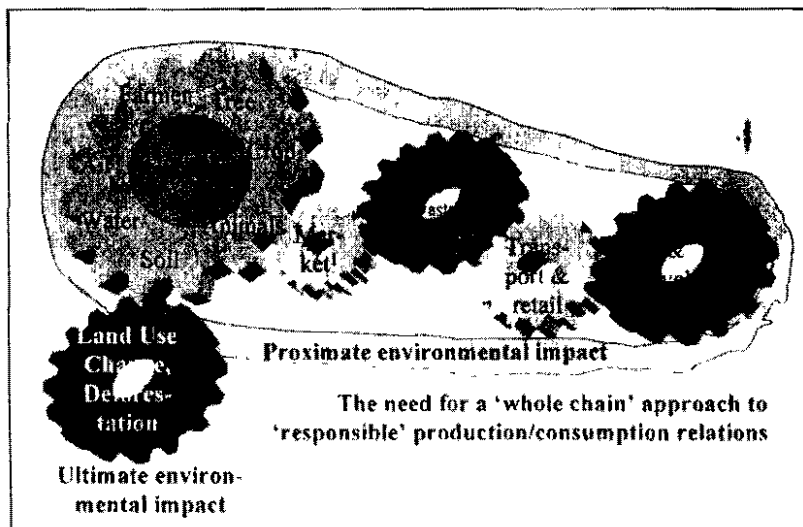


Figure 15. Sustainable management of belowground biota will require efforts within the agricultural production chain (including the feedbacks between (responsible) consumers and (sensible) producers that are grouped under an 'ecolabel' approach, as well as clarity and solutions for the 'ultimate' drivers of further conversion of remaining forests for agricultural use.



At the national scale, the CSM-BGBD Indonesia team may consider to start with four working groups for the initial stage of the project where the focus is on the survey.

Group A will focus on the selection of sampling windows and sample points, use GIS tools for describing land use history of the sites and establish the relations with the farmers managing the plots where we want to sample; this group will also conduct the interviews that help in deriving the  $I_{LUI}$  index at plot scale. A 'window master' will ensure that local contacts are maintained in an appropriate way, and that sampling efforts in the field are coordinated well

Group B will focus on the soil biota (with macro and micro as tentative subgroups, requiring different sampling methods)

Group C will be responsible for all other basic descriptors of the sampling sites: soil profile data, soil physical and chemical characterization, carbon stocks, greenhouse gas emissions and aboveground vegetation and biodiversity indicators

Group D will focus on the economical analysis of land use options in the windows and benchmarks, and on attempts to define the 'value' of BGBD for local land users linked to this value of land use per se. Policy dialogues will start early on, to sensitize the public debate on issues of CSM-BGBD, as soon as data from the survey will come in.

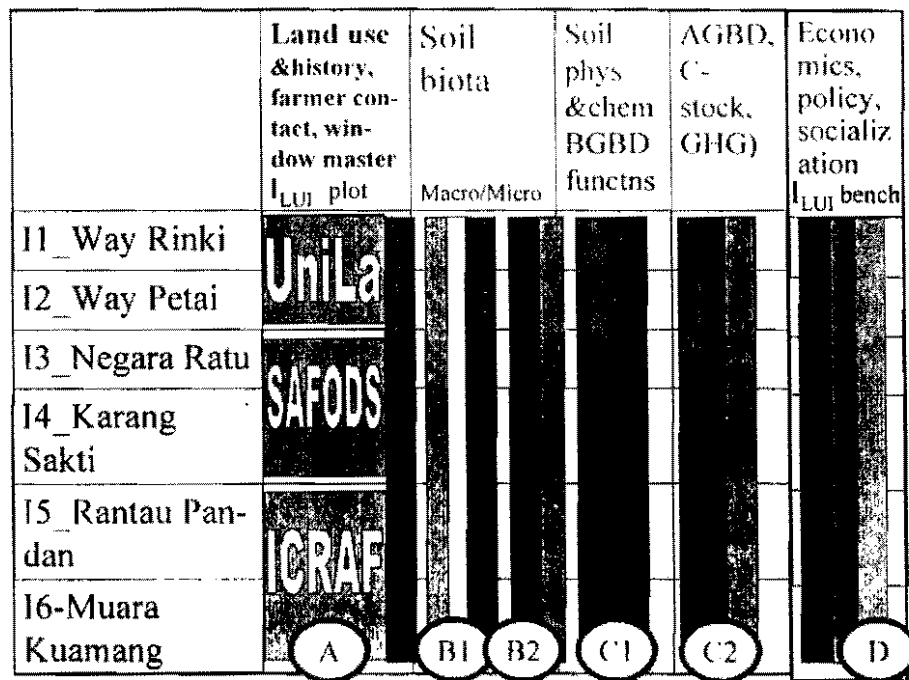


Figure 17. Diagram reflecting the responsibilities of the various groups for coherent sampling across all windows and sites, in order to allow subsequent data analysis to focus on the 'intensification hypothesis' rather than variations in method; for group A, however, the concept of 'window masters' is introduced to have a coordination level between all BGBD researchers and the local farmers.

Williams, S.E., Gillison, A.N. and Van Noordwijk, M., (2001). Biodiversity: issues relevant to integrated natural resource management in the humid tropics. ASB\_LN 5. In: Van Noordwijk, M, Williams, S.E. and Verbist, B. (Eds.) 2001. Towards integrated natural resource management in forest margins of the humid tropics: local action and global concerns. ASB-Lecture Notes 1 – 12. International Centre for Research in Agroforestry (ICRAF), Bogor, Indonesia. Also available from: <http://www.icraf.cgiar.org/sea/Training/Materials/ASB-TM/ASB-ICRAFSEA-LN.htm>.

The second step is to decide the amount and locations of the sample sites on each land use, using grid system or proportional sampling. Proportional sampling means one sample represents an area that the same as grid sampling, but for deciding process is not systematic. The main reason for deciding sample sites is accessibility.

## Collecting Data

### Data sources

Geographic information systems are essentially concerned with spatially referenced data. These data will normally be obtained from one or more of the following sources.

1. Existing maps. Any kind of maps can be used as sources of GIS data using digitizing process.
2. Aerial photographs. Aerial photographs can be used as a source of GIS data using manual analysis. To avoid error from the aerial photographs, it should be already rectified.
3. Satellite imagery. Satellite imagery such as Landsat image, SPOT image, IKONOS image, etc. can be used as a source of GIS data using digital image analysis. The image based remote sensing can provide near real time data; however, the availability of fine spatial resolution and near real-time data are still limited.
4. Field measurements. The primary data source of GIS is field measurement by collecting samples directly in the field.
5. Other GIS databases.

Associated with the spatial data are the non-spatial (attribute) data obtained from field observations, point sampling, census figures, etc. Input of non-spatial data to the GIS database is generally performed by typing at the keyboard. Many systems also accept data files imported from commercial database and spreadsheet software packages.

### Entering data

Before any spatial analysis or modeling operations can be carried out in a GIS, it is necessary to input the requisite data. The task of data input is generally the most time consuming; therefore, relatively the highest cost aspects of GIS implementation and operation. In particular, careful attention must be paid to quality control in order to ensure that the input data are the highest quality. Without quality control, the time and expense of data collection and input may be wasted (Weir, 1991).

The method of spatial data input depends primarily on the source of the data. The two principle approaches for inputting data from maps are digitizing and scanning.

### Data input by cartographic digitizing

The most usual method of converting existing maps into digital form is by digitizing. Using a digitizer, all features are recorded as a series of X and Y coordinates. Data input from aerial photographs is basically similar, with the exception that the third dimension is also recorded as a Z coordinate.

The process of map digitizing involves: (a) preparation of the map for digitizing, (b) recording of the map coordinates, (c) editing and correcting the data before storing in the map database, and (d) entering the associated attribute codes. Before starting digitizing, it is necessary to ensure that the source map is complete (including grid or graticule), correct, and free of physical damage (folds, tears, etc.). If feature labels are to be added during digitizing, the operator must also be provided with a list of the relevant codes. A digitizer records coordinates in units of millimeters (most digitizers can also record in inches if required). These coordinates are referenced to the X and Y axes of the table. During the digitizing, the progress of the work will be displayed on the monitor of the computer.

Base map preparation is an important things in GIS. The final maps of all the data measured in the field should be based on the base map as the absolute reference. The source of base map can be used includes topographic map, land use map, aerial photographs, satellite image, etc. Before starting to digitize the base map, the important thing is to prepare the map by making appropriate marks directly on the map document to identify the exact locations that will be digitizing. Remember that the information content on the base map is not as detail as the source map, but should be reduced or generalized. All information should be digitized and saved into a layer separately. Some benchmarks that will be easy to find out in the field should be clearly shown in the base map, such as road and river junctions, road and river curves, villages, etc.

Semivariogram analysis, kriging map and spatial maps are carried out using a geostatistical software (GS+) version 5.0 (Robertson, 2000) and then continued for the final maps using ArcView GIS 3.2 software (Hohl and Mayo, 1997). Semivariogram analysis will be used to evaluate the spatial variability of the properties, representing the relationship between the lag or any integral multiple of the sampling and semivariance. In this study, the best model in isotropic form will be used to fit the data to describe the variograms considering the lowest value of residual sum of square (RSS). Kriged map or spatial map of each variables will be computed subsequently using point kriging, by taking into account the data within the range we needed (McBratney and Pringle, 1997; Yanai et al., 2000; Syam and Jusoff, 2001). Kriged maps will be continued for spatial analysis using ArcView software. Kriged map will be exported and changed into ArcView format, registered and digitized separately based on the variability ranges of each variable. Each of the variable range should be saved as one layer. The final spatial maps can be drawn by overlaying some layers depending on the user's need.

## Output

Some means of output is required in order to present the results of GIS analysis to the system user, such as: hard copy output, i.e. a permanent copy, usually in the form of a printed spatial map; soft copy, in the form of a digital map on the computer monitor.

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## METHODS IN NEMATODE AND SOIL MICROBE RESEARCH FOR BELOWGROUND BIODIVERSITY ASSESSMENT

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### Introduction

Recently increased awareness to conserve and sustainable manage belowground biodiversity (BGBD) for sustainable development in the agricultural and other related sectors has been acknowledged. This is particularly due to widespread appreciation of the roles of belowground biota in ecosystems. As described by Swift and Bignell (2001), the activities of these organisms contribute significantly to the maintenance and productivity of agroecosystems by their influence on soil fertility and plant health. Despite this recognition, however, only a little is known, especially on their characterization and ecological functions. Similarly, soil and plant roots as environments for this diverse group of belowground biota, including nematodes and microbes, are still poorly understood. Assessment and evaluation of BGBD in different landscapes and land uses are therefore subjects of considerable importance.

Methodological aspects, including sampling, recovery, and identification, are very crucial in BGBD studies. For different experimental studies, a number of methods to assess nematodes and microbes have actually been available. It is important, however, to note that any selected method has to recognize at least two things, scientific justification and practical consideration. In the context of the Conservation and Sustainable Management of BGBD (CSM-BGBD) project, a workshop on methods for soil microbes is yet to be implemented. While outcomes of the limited workshop should serve as main references for microbial protocols, this paper is more or less a general discussion on procedures in nematode and soil microbe research for belowground biodiversity assessment. A quick review on molecular identification of microbes is also presented.

As already indicated, discussion in this paper focuses on methods in nematode and soil microbe research. Considering great variations of these organisms, a relevant question is on whether all of their taxonomic groups should be included. While the taxonomic diversity of nematodes is very high, that of soil microbes is even much more impressive. This inevitably leads to some selection of the organisms, particularly within soil microbes. Based on their functional significance, a number of microbial taxonomic groups have been selected to be investigated in the CSM-BGBD project (Sumberjaya technical workshop report, 2003). Under this recommendation, soil microbes are limited to bacteria, fungi and pseudofungi, with emphasis on certain functional groups such as mutualistic symbionts (arbuscular mycorrhizal fungi, ectomycorrhizal fungi and nitrogen-fixing bacteria), soil-borne pathogens and biological control agents (*Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp. and *Trichoderma* spp.) and some other ecologically important groups (ammonifiers, decomposers, denitrifiers, methanogenes, nitrifiers, etc.).

### Sampling

One early step in BGBD explorations is sampling. Nematodes and soil microbes are sampled in a stratified-grid system, in which 100 sample points will be included in each of the three windows comprising seven different land use systems, respectively. Sample units for nematodes and microbes are determined following the method described by S.P. Huang and J.E. Cares (personal communication). In each sample point, two vertically cross lines within two circles (3 m and 6 m radius, respectively) are drawn. Sample units include 12 soil cores taken to 20 cm in depth using a carbon steel tube. Four sample units are taken from the small circle, the remaining eight cores from the large

modified centrifugation sugar flotation method (Jenkins, 1964). By the method, the suspension is rotated at 3500 rpm for 5 min to discard the supernatant. The residue in the centrifuge tube is resuspended with sucrose solution (456 g/l) and rerotated at 1000 rpm for 1-2 min. Nematodes in the supernatant are then collected by a 37- $\mu$ m screen. The extracted nematodes are killed by hot water, and fixed by Golden solution (final in 3 % of formalin) (Hopper, 1970), and the suspension is adjusted to 15 ml. The nematode population is counted by randomly removing 1 ml from the solution, and the total number is calculated by the mean of three counts x 15.

#### Nematode identification and functional groups

Seinhorst's method (Seinhorst, 1959) is modified (Figure 2) to avoid picking up nematodes one by one for making high amounts of permanent slides. By this method, the nematode suspension is reduced to 3 ml, also added with 7 ml of Seinhorst I solution in a 5 cm diam Petri dish, and placed into desiccators at 43 °C for overnight. Being removed from the desiccators, the solution is dried at the same temperature for 4 h to reduce at least half volume on the second day. After being completed to the same volume (10 ml) with Seinhorst II solution, the dish is returned to the desiccators for overnight again. The process is repeated for three times (it is not necessary to add Seinhorst II in the last time), and the dish is maintained at the same temperature for at least 48 h. After this process the nematodes from the dish are mounted on slides. As many as 100 nematodes from the slides in each sample are randomly selected for identification to genus level by using a compound microscope (400-1000x). Composition of the reagents used in this method is presented in Table 1. The nematodes in genus level are then allocated into five trophic groups (bacterial and fungal feeders, plant parasites, omnivores and predators) based on description of Yeates *et al.* (1993).

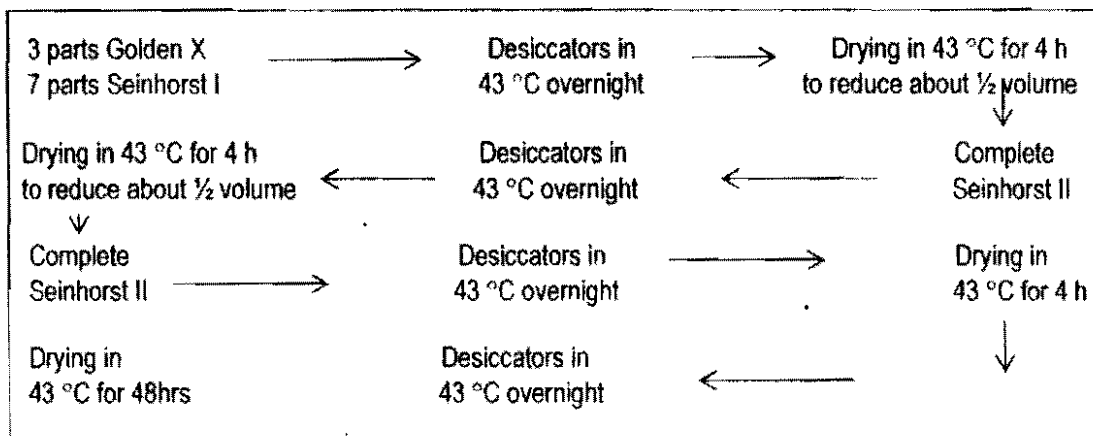


Figure 2. Scheme of the modified Seinhorst's method (infiltration of glycerin to high amount of nematode population). The desiccators are filled until about 1/3 of volume with 96% alcohol.

#### Indices and parameters

The data are then transformed into the following measurements with formulas described (Krebs, 1994; Magurran, 1988; Norton, 1978): absolute frequency, total abundance, relative abundance, trophic groups (Yeates *et al.*, 1993) (if one nematode have two types of feeding habits, its population number is divided by two for each one), species richness index ( $d = (S - 1)/\log N$ , where  $S$  = no. of genera, and  $N$  = total no. of nematodes), Simpson's diversity index ( $D_s = 1 - \sum(p_i)^2$ , where  $p_i$  = percent of genus "i" in the total abundance), Shannon-Weaver's diversity index ( $H' = -\sum p_i \log_2 p_i$ ), evenness of Simpson's diversity index ( $E_s = D_s/D_{s_{max}}$ , where  $D_{s_{max}} = 1 - 1/S$ ) and of Shannon-Weaver's diversity index ( $J' = H'/H'_{max}$ , where  $H'_{max} = \log_2 S$ ), trophic diversity index ( $T = 1/\sum(p_i)^2$ , where  $p_i$  = relative abundance of one trophic group), the ratios of fungivore/bacterivore (FF/BF) and of

standard protocol of DNA extraction involves grinding of the sample in a buffer, incubation, and extraction with phenol:chloroform to remove protein before DNA recovery by alcohol precipitation.

Table 2. Studies on soil microbial diversity employing molecular approaches (from Rondon *et al.*, 1999)

Habitat	Location (number of study)
Agricultural	Asia (1), Europe (3), North America (3)
Forest	Australia (2), Europe (2), South America (1)
Grassland	Europe (2)
Landfill	North America (1)
Pasture	Europe (1), North America (1), South America (1)
Peat bog	Europe (2)
Rainforest	North America (1)
Tundra	Asia (1)
Woodlands	North America (1)

Based on the genomic materials used or targeted, two types of molecular analysis are recognized. The first category utilizes PCR primers to amplify a single particular gene from the microbes. Ribosomal DNA (rDNA), the DNA that codes for the subunits of the ribosomes, is the most commonly targeted gene in the single gene approach. These genes occur as a multiply repeated cluster and consist of the genes for the ribosomal small subunit (SSU), the 5.8S subunit and the large subunit (LSU). The genes are arranged linearly in this order and are separated by two internal transcribed spacers (ITS). Each gene cluster is further separated by intergenic spacer (IGS) regions. The various ribosomal genes are very similar across greatly different life forms. They have evolved quite slowly (Duncan and Cooke, 2002). On the other hand, the spacers have evolved much more rapidly and, as a result, are much more variable, even among quite closely related organisms (Duncan and Cooke, 2002). Perhaps due to the complexity of the structure of ribosomal genes, the second type that samples and analyzes the whole genome have also been used as an alternative to the single gene method.

The single gene approach can be further divided into those employing primers selected for a specific taxon and those aiming to target a more general group of microbes. In general, primers often have to be designed to be specific for a particular exploration. As more information becomes available, the design of PCR primers also continues to advance. It remains, however, a delicate balance between generality that is needed so that important target organisms are not missed (for example in community analysis) and specificity to avoid being heavily contaminated by non-target organisms. Depending on the nature of the investigation, this can be facilitated among others by choosing the regions of the ribosomal genes to be amplified. Conserved rDNA regions are intended to determine relationships among all the major branches of life, whereas much more variable spacers should be useful to distinguish closely related organisms.

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soil formation (pedogenesis). Dindal and Wray (1977) noted that there are at least three main roles played by soil invertebrates including

- assisting the process of conversion of soil physical properties,
- playing a part in accelerating the decomposition rate of organic matters,
- sharing actively in humus formation.

Furthermore, Pankhurst *et al.* (1997), Linden *et al.* (1994) in Doube and Schmidt (1997) explained that soil organisms contribute to soil health in the form of

- controlling decomposition of plants and animals,
- controlling biogeochemical cycles,
- determining soil structure,
- determining changes of chemicals used in agriculture and pollutants.

An example in this case is earthworm. Lee (1985) in Pankhurst (1997) stated that this worms play important roles for reparation of soil fertility and structure repair with their activities. The importance of earthworms are as follows

- Resolving organic matter in soil
- Improving water holding capacity by soil particles
- Improving infiltration of ground water
- Improving soil aeration
- Improving root penetration into soil
- Improving activities of soil microorganisms

#### *Biocontrol agents*

Besides as decomposers, soil organisms can be used as biocontrol agent for plant pests and pathogens, both on the soil surface and plant roots. Besides the organisms themselves, utilization of metabolites from the organisms and interaction from various soil microorganisms have also been developed.

Individual exploitation of organism has been recognized; for example, utilization of certain fungi to control pest insects or to control plant pathogenic bacteria or fungi. Bacteria (Bt for example) as biocontrol agents for pest insects have also been commercially circulated with various trade names. Entomofagous fungi are not less effective than bacteria. Mostly the organisms used as biocontrol agents are soil organisms.

Metabolites of certain organisms can also be used to control diseases or pests. Some examples are antibiotic and/or allelopathic compounds released by various fungi. The fungi excrete compounds capable of inhibiting or even killing other organisms. This is also initially originated from soil.

Interaction of various fungi and/or bacteria can generate effect that can be used to inhibit growth of pathogenic fungi or bacteria. In the process of compost formation, the interaction happens. Side effects of the interaction are used to control pathogens. Results of this kind of research show that this can be accomplished (Djahuri *et al.*, 1997).

#### **Determinant Factors of Abundance of Soil Organisms**

Composition and abundance of soil organisms depend on soil types, physical and chemical conditions and management. Environmental conditions affect life of soil organisms. Microclimate, soil humidity, acidity levels, organic matter contents and existing vegetation can influence community of soil organisms (Clement and Pedigo, 1970; Aleinikova and Utrobina, 1975; Wallwork, 1975).

Various soil managements that are environment-friendly (minimum tillage, organic fertilization, etc.) generally can increase earthworm population. This matter is caused by the abundance of food (Doube and Schmidt, 1997). Mhatre and Pankhurst (1997) emphasized that relative abundance of soil organisms, for example nematodes, is determined by availability of food for the organisms. Bengtsson

- Adding soil organisms to accelerate decomposition processes (earthworm, EM). Earthworm could also improve community diversity of other soil organisms (Pankhurst, 1997).
- Other efforts (less synthetic fertilizers, pesticide, soil tillage, etc.).

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2. Tree-based land use (T = tree)
  - $T_e$  mixture tree (agroforestry), extensively managed
  - $T_m$  simple agroforestry, rather intensively managed
  - $T_s$  intensive monoculture plantation
3. Crop-based land use (C = crop)
  - $C_e$  extensive (R < 33 %), "bero" system, multiple cropping
  - $C_m$  rather intensive crop farming (R = 33-67 %), rather brief "bero" system
  - $C_i$  intensive crop farming (R = 33-67 %), managed brief "bero"
  - $C_p$  continuous crop farming (R > 67 %), very brief "bero"
4. Livestock production-based land use (A = animal production)
  - $A_e$  pasture without/low management of feed quality and quantity
  - $A_i$  pasture with management of feed quality and quantity

From the entire land use systems above, there has been agreement within the BGBD project to cover land use systems as wide as possible, starting from the most extensive to the most intensive systems. Thus, information about belowground biodiversity can be collectable from all existing land uses. Based on the condition of research location of the BGBD program, there will probably be seven classes of land use systems covering:

- Forest-based land use: natural forest ( $F_e$ ) and intensive production forest ( $F_i$ )
- Tree-based land use: extensive ( $T_e$ ) and intensive ( $T_i$ )
- Crop-based land use: extensive ( $C_e$ ) and intensive ( $C_i$ ), and
- Livestock production-based land use (A)

As an example, from surveys in some key areas assumed as representatives for Pakuan Ratu area, Lampung Utara, some dominant land cover groups are obtained. Land cover (found around villages of Karangasaki, Negara Jaya and Bimasakti) includes:

Acacia (*mangium*), "alang-alang" (*Imperata*), cassava, gallery forest, rubber monoculture, rubber - cassava (multi cropping), field rice, oil palm, "sengon", sugar cane, oil palm - cassava (multi cropping), maize - cassava (multi cropping), coconut and crop mixture.

#### Land Use Practices in Pakuan Ratu

Pakuan Ratu area in Lampung Utara represents a hilly to rather plane area, most of which is in the form of acid Ultisols soil experiencing damages at various levels (degraded soils) after it was left 'open' for decades. Land use system classes existing in Pakuan Ratu, based on combination between land use system classification and results of field observation are as follows.

No	Land Use System	Classification
1	Gallery forest1	$F_e$
2	Gallery forest2	$F_i$
3	Acacia, "sengon", coconut	$T_e$
4	Rubber, oil palm	$T_i$
5	Mixed crop, <i>Imperata</i>	$C_e$
6	Cassava, rice, suger	$C_i$
7	<i>Imperata</i>	A

**Characterization of Land Use System Intensity**

The BGBD Workshop in Sumberjaya proposed quantification of land use system intensity with an index calculated with the following formula (van Noordwijk, 2003).

$$I_{LUI} = \left( \begin{aligned} & \left( \frac{R_c t_c}{t_c + t_f} \right) (y_c) \left( 1 + \frac{1}{M_c} \right) \left( \frac{B_{h,c} + B_{f,c}}{B_c} \right) \left( 1 + \frac{N_{fertilized,c}}{n_c B_c} \right) \\ & + \left( 1 + \frac{W_{irrigated,c}}{10 B_c / w_c} \right) \left( 1 + \sum_i \frac{P_{used,c,i} T_{1/2,i}}{p_i} \right) \frac{L_c}{100} \left( 1 + \frac{E_{utilized,c}}{e_c B_c} \right) \\ & + \left( \frac{R_f t_f}{t_c + t_f} + R_p \right) \left( 1 + \frac{1}{M_f} \right) \left( \frac{B_{h,f} + B_{f,f}}{B_f} \right) \left( 1 + \frac{N_{fertilized,f}}{n_f B_f} \right) \\ & + \left( 1 + \frac{W_{irrigated,f}}{10 B_f / w_f} \right) \left( 1 + \sum_i \frac{P_{used,f,i} T_{1/2,i}}{p_i} \right) \frac{L_f}{100} \left( 1 + \frac{E_{utilized,f}}{e_f B_f} \right) \end{aligned} \right) (1 - R_{rel}) S$$

in which

- t** = length of cropping or fallow period in a typical rotation [year] (Interview the farmer for plot history)
- B** = (final) total biomass of a crop or fallow vegetation [Mg ha<sup>-1</sup>] (destructive biomass sample, use of allometrics for woody perennials)
- B<sub>h</sub>** = (cumulatively) harvested part of the biomass of a crop or fallow vegetation [Mg ha<sup>-1</sup>] (interview the farmer for plot history)
- N<sub>fertilized</sub>** = the amount of plant nutrients (N + P + K) added to the field as external fertilizer (In inorganic or organic form, the key is that it is derived from outside of the 'system' under consideration) [kg·ha<sup>-1</sup>] (Interview the farmer for plot history)
- n<sub>c</sub>** = typical nutrient (N + P + K) concentration [kg Mg<sup>-1</sup>] (for crops there are databases that we can use, occasionally we may need to sample ourselves)
- W<sub>irrigated</sub>** = amount of water provided by irrigation during one cropping year [mm] (interview the farmer for plot history)
- w** = water use efficiency of the crop, or biomass production per unit of water transpired [kg / l] (the factor 10 is required to make the term dimensionless) (database to construct lookup table for local climate and C3 versus C4)
- E<sub>utilized</sub>** = sum of fossil energy used for all soil tillage and mechanized harvest operations [MJ ha<sup>-1</sup>] (interview the farmer for plot history)
- e** = typical energy content of crop biomass [MJ Mg<sup>-1</sup>] (database for lookup table based on harvested product)
- P<sub>used</sub>** = total amount of active ingredient of pesticides used [kg ha<sup>-1</sup>] (interview the farmer for plot history)
- T<sub>1/2</sub>** = half-life time of the active ingredient [year] (database)
- p** = a biological impact rating of the various active ingredients [kg year ha<sup>-1</sup>] ('expert' rankings?)

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**SOCIALIZATION OF THE CONSERVATION AND SUSTAINABLE  
MANAGEMENT OF BELOWGROUND BIODIVERSITY (CSM-BGBD) PROGRAM IN INDONESIA:  
HOW TO ENCOURAGE THE COMMUNITY'S PARTICIPATION?**

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**Introduction**

The definition of conservation has some relevance to the meanings of impartiality, strategy, and dynamism. Conservation is the management of natural life to benefit the present generation fully and sustainably, and to conserve its potential to guarantee the next generation's aspiration and needs (Wiratno *et al.*, 2002). The awareness of conservation is low due to the limited knowledge of the long-term benefit of below ground biota in communal living. Conservational effort of belowground biota will give long-term benefit. Socialization is, therefore, necessary.

Many theories of behavior, such as S-R model (Stimulus – Response) and C-R model (Challenge – Response) may be applied as a base to recognize changes in behavior. They state in principle that behavior is a result of some responses of observed individuals, which will stimulate him/her later both internally and externally. Behavior of S-R and C-R models is the result of one's external stimuli. Changes in behavior may be caused by one's inner-self or outer factors. Behavioral changes based on one's inner-self consciousness are more permanent (Ndraha, 1991).

The concept of participation has various and wide definitions. Often is the concept of participation measured and defined as an involvement of community in an activity. The word "involvement" is actually the essence of an understanding of participation. Participation can also be defined as one's mental and emotional involvement in a particular group, which encourages him/her to make contributions to the group, in an effort to achieve its goal, and to be responsible as well.

To reach the context of active participation, understanding and "consciousness level" are essential so that one will participate in an "aware-willing-able" way. To participate, one requires conceptual ability and potential to contribute. Without them, he/she will find it difficult to participate or perform himself/herself as expected. The ability to participate implies that the result of a particular process related to stimulus or motive, manner, intelligence, knowledge, skill, and utilization of methods, means, and instruments, becomes a completeness of doing (Ginting, 2001).

**Communal Participation in CSM-BGBD Program**

Communal participation in the Conservation and Sustainable Management of Belowground Biodiversity (CSM-BGBD) program will take place if the CSM-BGBD contribution to the community is real. The objective of CSM-BGBD in Indonesia is still beyond community's perception because they have not had the advantage of it directly. Therefore, it is necessary to make an effort to enable the community to get benefit from conservation and belowground biota management activities.

Opportunity to participate should be supported by some factors coming from both inside and outside the community. Inner supporting factors include (a) having an opportunity to participate in an activity, (b) possessing an ambition to achieve goals, (c) willing to join an existing activity, (d) keeping trust and hopes and (e) having reciprocal cooperation and adjustment between the community and program agents (Mikkelsen, 1999). On the other hand, participation is closely related to empowerment, because participation actually empties into the emergence of inner ability, so that participation has an active meaning. In the context of development, "participation" frequently serves as justification for program or activity siding with the community and communal involvement in which the community basically remains as an "object"; hence, obviously this is not a positive sense of the term.

There are several details to consider in working on communal participation with regard to socialization and escalation of communal participation in CSM-BGBD.

- Participation serves as a stimulus in the community to encourage responses.
- Participation performs a motivation for the community to develop sustainable appropriate conduct.
- Participation benefits the related community directly.
- Participation depends on felt need in the community.

### Policy Aspect of CSM-BGBD

A program will work applicably and efficiently in its process if there is a support of policies. In policy system, there is a harmonic reciprocal relationship among the three elements: public policies, policy stakeholders, and policy environment (Dunn, 2000). CSM-BGBD programs require policies' support for introducing the community to their importance.

The first element to apply the policy correctly is the presence of public policies' support. According to Dunn (2000), public policies' support is a series of choices showing, more or less, a relationship made by government officials, which may serve as a decision on flora and fauna protection and conservation. In formulating policies, it is likely to side with an issue that is potential to attract conflicts and the actual condition. The available policies are usually the results of definition conflicts of policy problems.

Furthermore, it is also important to consider the policy stakeholders. Policies depend upon the policy stakeholders' involvement pattern, in which individuals and groups make contributions to the policies due to the actual mechanism influencing and being influenced by government resolution. Policy stakeholders are those who involve directly or indirectly in appreciating a government resolution, such as citizens, NGOs, political parties, government agents, leaders, etc. The stakeholders often comprehend the same information on policy environment differently.

The third element, policy environment, is a particular context in which events around the issue of policies affect and are affected by policy makers and public policies. Consequently, policy system covers a dialectical process, meaning that objective and subjective dimensions of policy makers are not separable in practice. Policy system is a product established through sensible and rational choices of the policy stakeholders.

Public support means that policy enforcement will be more effective and efficient in socializing the program. The involvement pattern of the policy stakeholders is actively related to their capacity as subject of the program's planning, execution, and evaluation. Policy environment suggests the appearance of strategic issues coming from a specific pattern of CSM-BGBD performance. A series of events can serve as a reflection of policy execution.

One of the efforts to enhance CSM-BGBD program is through a policy advocacy activity. The utilization of advocacy method is to distribute CSM-BGBD's ideas and goals and through the utilization of information relevant to actual policies, to claim rational knowledge based on reasonable arguments about possible solutions to policy problems. Policy advocacy is a way to make normative statements, instead of giving prescriptions, commands, orders, and so on.

The government, in executing policies, has flexibility or transitory regulations, while the government's competence in making policies has a strong and binding legal aspect. Stewart (1999) mentioned several tools for the government to implement its policies (Table 1).

By observing Table 1 below, it is confirmed that the government in carrying out policies has a very specific scope especially for the achievement of goals. Speaking of strategy and policy means relating the three tools to obtain an appropriate objective without leaving the concept of efficiency and effectiveness behind in utilizing resources. The achievement of policy goals is identical to the establishment of effective and efficient policies. Accordingly, all social strata in the community will respond to the policies' objective in executing the CSM-BGBD program.

## BIOPHYSICAL PROPERTIES OF THE CONSERVATION AND SUSTAINABLE MANAGEMENT OF BELOWGROUND BIODIVERSITY (CSM-BGBD) PROJECT SITES IN INDONESIA

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### Introduction

The Conservation and Sustainable Management Belowground Biodiversity (CSM-BGBD) Project in Indonesia will be conducted in three benchmark areas in Sumatra Island. Two benchmarks, Sumber Jaya benchmark (SJ) and Pakuan Ratu benchmark (PR) are located in Lampung Province, while the other benchmark will be located in Jambi Province (Figure 1). The Jambi benchmark also consisted of two sites, Rantau Pandan and Muara Kuamang. The benchmark areas represent the ecological zone of Sumatra Island, which consisted of mountainous, piedmont, peneplain and swampy areas. Sumber Jaya is located in the mountainous areas, while Rantau Pandan in the piedmont areas, both Muara Kuamang and Pakuan Ratu are located in peneplain region.

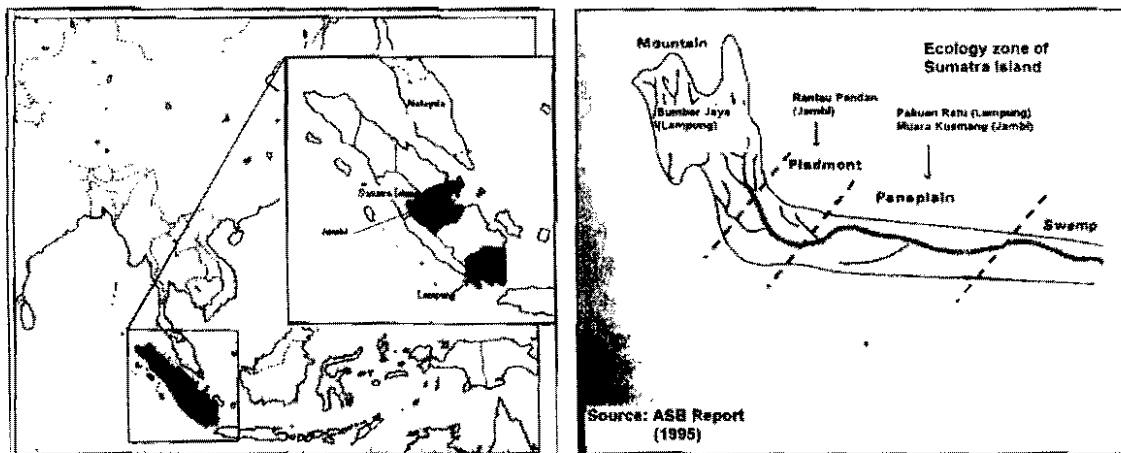


Figure 1. Location map of Sumatra Island, Lampung and Jambi (A) and position of the benchmark areas from the ecological zone view point of Sumatra Island (B).

In addition to this, Lampung Province represents the areas with high population density and high - pressure on natural resources, in contrast with Jambi Province, which represents low population density. Population census in 2000 showed that the population of Lampung was 6.7 million while Jambi had 2.4 millions population. Lampung province has the highest population density in Sumatra Island, 191 population per km<sup>2</sup>, while Jambi has the lowest population density, 45 population per km<sup>2</sup> (BPS, 2003).



On field level observation, Afandi *et al.* (2002b) reported that there were many shrubs areas which grew along the river areas ("riparian"), which effectively prevented soil loss. Afandi *et al.* (2002a) also reported that although the farmer applied "clean - weeded" management in coffee areas, the soil loss based on plot scale measurement was very low, with the maximum of 22.7 t/ha.

#### *Pakuan Ratu benchmark*

Research on BGBD Project in the middle area of Tulang Bawang Watershed will be located around Way Kiri River with longitudes between 104°50' - 105°00' (East) and 4°25' - 4°30' (South).

#### Topography and geology

The main landform of the Pakuan Ratu benchmark area is acid tuff plain with undulating (3 – 8%) to rolling (8 – 16%) slope classes, and 25 – 100 m a.s.l altitude. Alluvial landform occupies a small part of this area, particularly along meandering belt.

According to Gafoer *et al.* (1993), the most part of the Pakuan Ratu area is located in the Kasai Formation (QTK), and the small part is in the Recent Alluvium (Qa). The Kasai Formation, which was previously named the Upper part of Palembang Zone (Bemmelen, 1949), consists of conglomerate and quartz sandstone, tuffaceous clay stone containing silicified wood with pumiceous tuff and lignite intercalations. Aggradation processes, which began in the early Tertiary Period until the early Quaternary Period filled the geosynclinal of Sumatra by sedimentary rock materials (Palembang zone) and formed widespread of peneplain in South Sumatra. The upper part of Palembang zone or Kasai Formation ( $\pm$  150 m thick) was deposited overlying the Muaraenim Formation (Tm<sub>pm</sub>) during Pliocene Epoch (late Tertiary) until Pleistocene Epoch (early Quaternary). The Recent Alluvium, which was accumulated in the Holocene Epoch, consists of boulder, gravel, sand, silt, mud, and clay. Those materials can be found along meandering belt of Way Besai River. Both of the two Formations belong to acid rock materials.

#### Climate

Rachman *et al.* (1997) reported that the annual rainfall was around 2500 mm/year with average annual temperature of about 26.8°C. The rainfall pattern in this area is shown in Figure 2.

#### Soil

According to the Land Unit and Soil Map of the Baturaja Sheet 1:250.000 (Hikmatullah *et al.*, 1990), there are several classes of soils (Great Soil Group Category, based on USDA, 1987) in the Pakuan Ratu benchmark area. Soils in the acid tuff plain were dominated by *Hapludox* and *Dystropepts*, while soils in the floodplain of meandering river (alluvial landform) are dominated by *Tropaquepts*, *Dystropepts*, and *Fluvaquents*.

#### Land use

Several land use types could be found in the Pakuan Ratu benchmark area in later years, such as secondary forest, fresh water swamp forest, riparian forest, mixed perennial crops, shrub and bush, upland crops, and grassland. There are some big private and national estate plantations, such as sugarcane plantation, oil palm plantation, pineapple plantation, and rubber plantation.

Table 1. Climate data for the Rantau Pandan benchmark area (Rachman *et al.*, 1997).

Parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Annual
Rainfall (mm)													
Tanah	339	273	230	353	171	109	115	181	191	217	336	411	2926
Tumbuh*													
Rantau	302	234	234	317	236	147	121	209	165	269	280	343	2898
Panjang*													
Bangka*	313	268	268	299	245	142	147	206	170	297	345	389	3146
Temp (°C)													
Average	29.1	28.6	28.6	29.1	28.7	29.4	27.1	29.4	30.4	28.9	28.8	29.0	28.9
Stand. Dev	0.30	0.30	0.30	0.15	0.60	0.40	0.20	0.10	0.05	0.10	0.05	0.25	0.23
Max	33.5	32.5	32.1	32.9	32.5	32.8	28.1	32.7	34.8	33.9	33.2	32.9	32.6
Min	24.6	24.8	25.0	25.3	24.9	25.9	26.0	26.0	25.9	23.8	24.3	25.1	25.1
ETP (mm)	112	123	138	134	120	117	114	127	127	117	97	124	1450
Radiation (MJ m <sup>-2</sup> day <sup>-1</sup> )													
Average	15.2	15.6	16.0	15.3	14.3	13.6	13.9	14.7	15.5	15.8	15.2	15.1	15.0

\*Climate Station

### Soil

Tropofluvents were found in the floodplain along the Bungo river with slope of less than 1% and contributed 12% of the whole area. Three types of soils in the sub group category occupied 88% of the whole area and distributed in five land units, and they were found in the hilly area of this benchmark. Those soils are *Oxic Dystropepts*, *Typic Dystropepts*, and *Lithic Dystropepts*. Soil solum varies between shallow and very deep. *Lithic Dystropepts* represent soils with shallow soil solum, while *Oxic and Typic Dystropepts* represent soils with deep to very deep soil solum. All of soils in the benchmark area have low base saturation (<50%) in all subhorizons between depths of 25 cm and 100 cm, or between 25 cm and a lithic contact. CEC of soils are generally low, particularly in the *Oxic Dystropepts* that have CEC clay of less than 24 cmol(+) kg<sup>-1</sup>. Soil with low CEC clay shows the domination of kaolinite in the clay fraction.

### Land use and farming systems

van Noordwijk *et al.* (1995) had identified ten kinds of land uses found in this benchmark area ie: 1) natural forest, 2) community - based forest management, 3) commercial logging, 4) rubber agroforests, 5) rubber agroforests with clonal planting material, 6) rubber monoculture, 7) oil palm monoculture, 8) upland rice/bush fallow rotation, 9) cassava/Imperata rotation, and 10) wetland rice fields.

### Muara Kuamang Window

Geographically, the Muara Kuamang window area is sited in the latitude between 102°10' - 102°20'E and 1°30' - 1°40'S. This area represents the penepain zone of Sumatra. There are several streams as the tributaries of Batanghari river i.e. Bungo Tebo river, B. Senamat river, B. Pelepat river, and Kuamang river. Kuamang village is in the sub - district of Pelepat and represents the local farmers (Jambi ethnic group).

### Geology

Rachman *et al.* (1997) reported that there were two kinds of landform found in this benchmark area, i.e. floodplain along the streams and rivers and undulating to rolling penepain. According to Geological Map of Sumatera 1:2,500,000 (Rosman and Dai, 1979), this windows area is located in the recent volcanic rock materials. Geological Map of Muarabungo Quadrangle (Simanjuntak *et al.*, 1991) gives information in more detail. This area particularly consists of the Kasai Formation (QTK) and Muaraenim Formation (Tmpm). Rock materials in the Kasai Formation were accumulated from the late Tertiary period (pliocene epoch) until early Quaternary period (pliocene epoch), and consist of conglomerate, quartz sandstone, tuffaceous

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