博士論文

Research on Synecoculture: Focusing on products and soils from a system-level perspective

（協生農法の研究：システムレベルの観点から産物や土壌に着目して）

Kousaku Ohta
太田 耕作

Ph.D Thesis (2022)

令和４年度　博士論文

Research on Synecoculture: Focusing on products and soils from a system-level perspective

（協生農法の研究：システムレベルの観点から産物や土壌に着目して）

Laboratory of Sustainable Agriculture, Department of Global Agricultural Sciences,
Graduate School of Agricultural and Life Sciences,
The University of Tokyo
(1-1-1, Yayoi, Bunkyo-ku, Japan, 113-8567)

東京大学大学院 農学生命科学研究科 農学国際専攻
国際植物資源科学研究室

Kousaku Ohta
太田 耕作

Supervisor: Kae Miyazawa
指導教員：宮沢 佳恵

Preface

Since I was a child, I have always wanted to make the world a better place. I have grown up looking for better means to achieve this goal. In the process, I chose to study sports science as my undergraduate degree because I wanted to study human performance in depth. Although the sports science methodology was very advanced in terms of elemental aspects such as strength training methods, there was a gap between the synthesis of these methods and improving the performance of the whole person. This is because the phenomenon of human performance is not a simple collection of elements.

I studied life sciences from this perspective during my master's program. Living organisms are also not a simple set of elements. To understand life, we must consider the whole, which is lost when we separate it into elements.

One of my motivations for doing this research of a new method of agriculture, Synecoculture, is to understand and manage agriculture well, which is also a complex system, not only by breaking it down into its elements, but also as a whole. Of course, one of the motivations was the sense of crisis over environmental, health, and food issues that worsen year by year. Synecoculture was formalized as a possible alternative to conventional farming methods, aiming to combine biodiversity and food production. I would like to devote myself to further contribute to society and this planet through this research.

Acknowledgments

First, I would like to express my deepest gratitude to my supervisor, Associate Professor Kae Miyazawa, for allowing me to conduct this research in this doctoral program and for her guidance and support. Her consideration allowed me to do this research very freely. I would also like to thank Professor Kazuhiko Kobayashi for his great advice. I would also like to thank Dr. Masatoshi Funabashi of Sony Computer Science Laboratories, Inc. (Sony CSL) for his advice and great support for this research. I also thank Associate Professor Shoji Oda of the Laboratory of Genome Stability at the University of Tokyo, Tatsuya Kawaoka, Godai Suzuki, Tomoyuki Minami, and Tanoy Debnath of Sony CSL, for their contribution in this research. I also received the contribution of Tsuyoshi Takeshita of the Laboratory of Plant Life System, The University of Tokyo. The other members of the Laboratory of Genome Stability also helped my research.

I am also very grateful to Dr. Shigeho Tanaka of the National Institute of Health and Nutrition for his help in measuring human activity levels. With the help of Mirai Shirakawa, I also received the cooperation of the subjects.

In addition, I would like to thank Yoko Honjo and Yuji Kawamura of Sony CSL, and Kei Fukuda and Satoru Okamoto of Synecoculture Association for their invaluable help in implementing our practices at the Roppongi Rooftop Farm. The use of the Roppongi rooftop was made possible with the support of Mori Building Co., Ltd.

I also thank Jordan White Springer and Arnold Ross Falvo who checked the English when I wrote the original paper (Publication C).

I also thank my great colleagues at the Laboratory of Sustainable Agriculture and Sony CSL Synecoculture Project for their great advice and help. I also thank Sony CSL, Synecoculture Association, SynecO, Inc., and Takashi Otsuka and his colleagues at Sakura Shizenjyuku The Global Nature Network for their cooperation.

This research was made possible by the financial support of Sony CSL.

Finally, I would also like to express my deepest gratitude to my beloved family, my invaluable friends, and all involved parties for their support during my seven years in the doctoral program, which was also a financial and mental challenge. During the past seven years, two of my grandfathers and one of my grandmothers have passed away. I would like to dedicate the safe writing of this thesis to them as well.

November 2022

Kousaku Ohta

Table of Contents

Abstract

Chapter.1 General Introduction

As monoculture cultivation using tillage, fertilizers, and pesticides in conventional farming methods has become a major burden on the global environment, the development of new methods of food production is an urgent issue. Synecoculture, proposed in 2011, is a comprehensive approach to solving the food-environment-health trilemma, and the number of people practicing it has been increasing in recent years. Synecoculture is characterized by the production of food through the management of the entire ecosystem by mixing and densely growing many plant species with no tillage, no fertilizers, and no chemicals. While there are examples of its practice and suggested effectiveness in developing countries where fertilizer and organic resources are scarce, there is little academic verification of its effectiveness. In this study, I examined the effectiveness of some aspects of the Synecoculture method mainly by comparing the quality of its products with those of conventional farming methods, and the effect of this method itself from a systems-level perspective. I will also discuss the effectiveness of verification methods based on a systems-level approach. The purpose of the research is to obtain knowledge and methodology that contributes to sustainable agricultural practices through the investigation of Synecoculture as one of the counterparts to conventional farming methods in terms of complexity.

Chapter.2 Comparison of Synecoculture products and conventional farming products

A comparison of arugula (*Eruca vesicaria ssp.sativa*, "ルッコラ" in Japanese) and Bancha (Japanese tea) grew on a Synecoculture farm and on conventional farms was conducted. In arugula, analysis of fatty acid composition suggested that arugula grown on Synecoculture farm had more antioxidant activity. A similar trend was observed for the Bancha. In Bancha, the metabolomic analysis revealed components that characterize each farming method. Vitamin B6-related compounds were detected as components that characterize Synecoculture Bancha. In addition, sensory evaluation tests of Bancha were conducted, and consistency was found between the taste evaluation and the trend inferred from its characteristic ingredients. In particular, Synecoculture Bancha was superior to conventional farming Bancha in terms of abstract taste, rather than simple tastes such as umami and sweetness. In addition, in an experiment to examine whether human consumption of these two types of Bancha causes changes in activity, it was observed that the effects of the two types of Bancha were different. These results connect agriculture's environmental impact with the product's health effects and suggest the

importance of examining the long-term effects of growing conditions on the environment and human health from a sustainability perspective (referred to as planetary health or one-health).

Chapter.3 The effectiveness of subjective evaluation by humans

Based on the theory of Synecoculture, I implemented Synecoculture in an urban area, analyzed the diversity and activity of soil microorganisms and soil chemistry, and conducted a subjective evaluation of the ecosystem by humans. The diversity and activity of soil microorganisms were higher after two years than after one year of implementation, confirming the effectiveness of the managing method based on Synecoculture in improving the soil in urban areas. Comparing these data with the subjective evaluation of the ecosystem by humans, a relationship was found between the diversity and activity of microorganisms and human evaluation two years after the practice. This result suggested that human subjectivity can be used as an indicator for evaluating an ecosystem, if properly trained to improve accuracy.

Chapter.4 Ecosystem Navigation

Augmented ecosystems, including Synecoculture, are managed to increase biodiversity and ecosystem function while observing complex ecosystems, but to do so, it is necessary to make appropriate assessments of ecosystems and learn more about their current conditions. In this chapter, I analyzed the effects of three operations (introduction of useful species, elimination of naturally occurring species, and abandonment) on two plots in an urban area using various indices. By classifying the commonalities and uniqueness of the two farms in the analysis, I was able to extract useful evaluation indicators for a complex and open ecosystem. This suggested that with the support of big data and ICT, it is possible to evaluate complex open ecosystems in detail using less expensive analytical methods.

Chapter.5 General Discussion

In this study, I have identified what kind of analysis is effective in detecting differences in complex ecosystems such as Synecoculture at various levels of hierarchy, from differences in single components of products to differences at the system level that are derived comprehensively from multiple components of products and multiple indicators such as soil data and human subjectivity. The study clarified what types of analyses are able and effective in detecting differences in complex ecosystems such as Synecoculture farms. In particular, the effectiveness of subjective evaluation by trained

people showed the possibility of using human subjective evaluation with objective data background not only for Synecoculture, but also for environmentally friendly agriculture and other farming methods. Further analysis of the relationship between scientific analysis and complex human cognition is expected to enhance the effectiveness of human evaluation in assessing sustainable agriculture and the ability of people to observe nature through feedback from objective analysis. These efforts are expected to contribute to the realization of sustainable agriculture by increasing the accuracy of system-level assessment of ecosystem functions.

1: General Introduction

1-1 Background

There are a wide range of issues that humankind is called upon to solve in modern society, but there are areas in which the natural sciences should be particularly involved: food, health, and environmental issues. These are closely interacting with each other, and each is not a completely independent issue; this is called trilemma [Tilman, et al. 2014]. One of the human activities that are deeply involved in all of these is agriculture.

From the dawn of agriculture, which is said to be 10,000 years ago, to the present day, conventional farming methods, represented by monocultures using tillage, fertilizers, and agrochemicals, have placed a heavy burden on the global environment. These three technologies of tillage, fertilizers, and agrochemicals are themselves a burden on the environment, and at the same time, because they use fossil fuels, they consume resources and emit greenhouse gases, creating a double burden on the environment. It is unlikely that the current system of agriculture based on these technologies can be sustainable [IAASTD 2008].

It is also said that the world's sixth mass extinction is occurring in the Anthropocene [Lewis and Maslin 2015], including reports on the mass extinction of insects [Sánchez-Bayo and Wyckhuys 2019] and vascular plants [Pereira et al. 2010]. Some researchers have predicted that the continued decline in biodiversity will eventually trigger a rapid global ecological collapse at some point [Barnosky, et al. 2012]. Under these circumstances in a world with the continuous increase of human population, there is an urgent need to transform agricultural activities into sustainable practices. The development of new agricultural technologies that reduce the burden on the environment are all being actively pursued around the world.

1-2 What is Synecoculture?

One such technology in agricultural practice is Synecoculture or synecological farming (協生農法 in Japanese), which was proposed by Masatoshi Funabashi in 2011 [Funabashi 2011]. For the sake of convenience, this will be referred to as Synecoculture in this thesis. It is a comprehensive approach to solving the food, environment, and health trilemma [Funabashi 2018]. Synecoculture is characterized by the use of no-till, no fertilizers, and no agrochemicals to produce food while managing the entire ecosystem by mixing and densely growing many plant species.

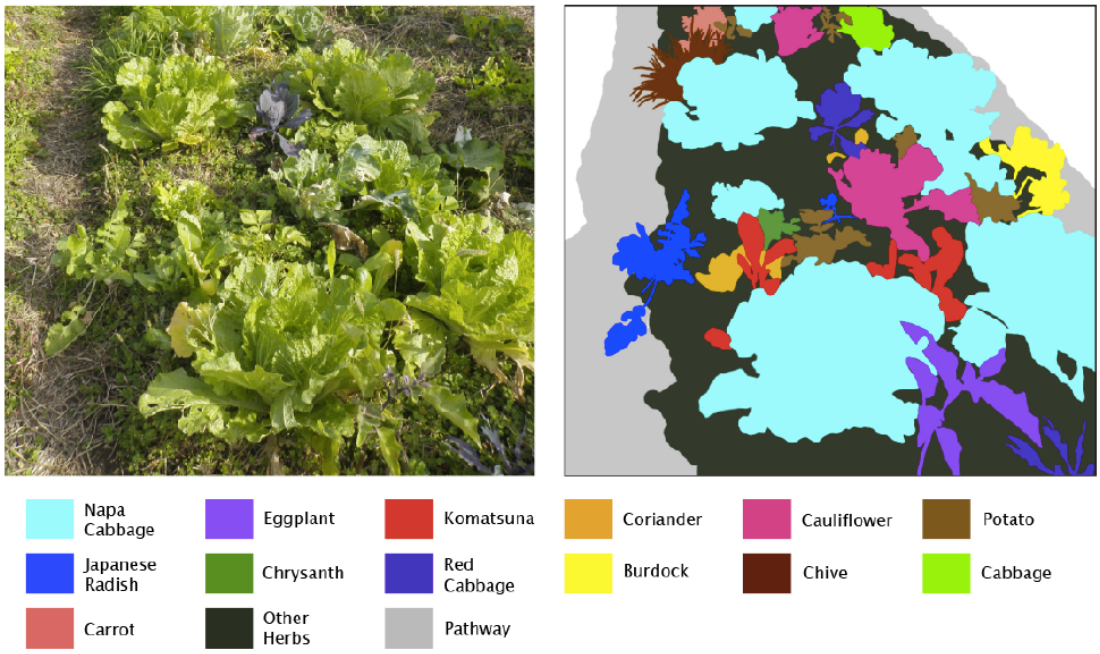


Figure 1. Example of Synecoculture field (Ise City, Mie Prefecture) [Funabashi 2016a]. In contrast to conventional farming, in which a single species is cultivated with a certain distance between individuals, Synecoculture cultivates a mixture of many species in a small plot, just as in the natural state. The left photo shows a field, and the right shows the different species of vegetables by color. The figure is the same as in the cited reference.

There are several other agricultural methods, including permaculture, natural farming, natural cultivation, radical carbon farming and agroforestry, all of which aim to produce food with less tillage, fertilizer, and agrochemicals and utilize natural ecosystems. However, Synecoculture is characterized not only by the elimination of the three elements (tillage, fertilizer, and agrochemicals), but also by the enhancement of biodiversity and the production of food in an ecological optimum. The goal is to increase biodiversity and overall ecosystem functioning beyond the natural state, and ecosystems in this state are referred to as augmented ecosystems.

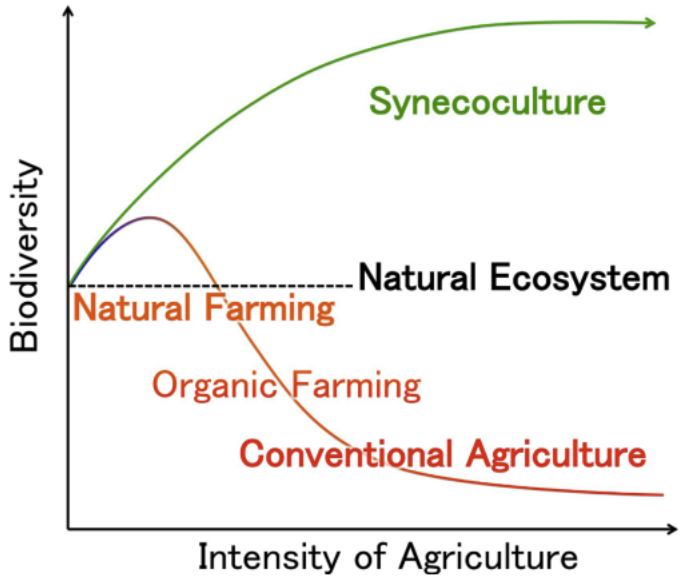


Figure 2. Intensity–biodiversity relationship of open-field culture [Funabashi 2016b].

Synecoculture aims to increase biodiversity while increasing productivity. This is a unique approach that aims to maximize biomass production at the community level in the context of ecological optimum, which is fundamentally different from conventional agricultural methods that focus on the plant species that humans want to produce and grow target plants through physiological optimum.

When beginning Synecoculture, if the land has been abandoned or originally had numerous plants growing in their natural state, all or some of them may be eliminated first. Then, if necessary, the initial conditions are adjusted to suit the purpose of the farm by, for example, creating ridges.

Various types of fruit trees are then planted and around them various types of vegetables and herbs are introduced by seedlings or seeds. Plant species are freely selected by the practitioner according to the purpose of the farm and environmental conditions, etc. Basically, practitioners aim for the soil of the production area to be completely covered with dense vegetation when viewed from above.

After that, irrigation is allowed depending on the situation, but basically practitioners let them grow. As the plants grow, they will thin-harvest the useful plants and manage the weeds. In other words, unlike conventional farming, the same type of crop is not harvested in large quantities at once, but a variety of plants are harvested gradually and frequently.

Afterward, a variety of vegetables and herbs are introduced again by seedlings or seeds in the vacant areas. These management practices need to be done at an appropriate frequency and intensity to increase biodiversity. This method itself is not reproducible in the first place, as the actual work is highly varied depending on the farm's environment, objectives, and practitioners. While there is a common direction of qualitatively moving complex ecosystems in the direction of higher biodiversity and ecosystem function, the methods vary widely. In other words, when we want to investigate Synecoculture farming methods, we need to take a large qualitative framework for each case.

What seeds are introduced, in what arrangement, and at what timing are the key techniques for successful practice. In addition, mowing and weeding are permitted for the purpose of increasing the biodiversity of the field. These can be done in a variety of ways depending on the conditions of the field and the goals of the practitioner. In other words, when practicing Synecoculture, one must consider the environmental conditions of the field, the species composition, and the stages of ecological succession, to strike a balance between the crop one wants to produce and an efficient increase in biodiversity, and one must determine and apply the appropriate methods to achieve this.

1-3 Synecoculture Achievements and Current Status

Synecoculture has been practiced by an NGO in Burkina Faso, Africa since 2015 and has reportedly achieved sales 20 times the GNI per capita [Tindano and Funabashi 2017]. This has been received with astonishment locally, and six international symposiums on Synecoculture have been held in Sahelian countries so far, and the practice is spreading to neighboring countries. Other examples of non-African practices can be found in China, Ecuador, and other countries [Human Augmentation of Ecosystems UniTwin UNESCO Complex Systems Digital Campus e-Laboratory][SynecO].

In Japan, Sony Computer Science Laboratories, Inc. is promoting this method [Sony CSL]. An increasing number of citizens are engaging in it as can be seen on social networking services, but as far as the author knows, there are no professional farmers practicing it yet.

1-4 Purpose of this study

This study focuses on Synecoculture, which has been proposed as a sustainable agricultural method that could contribute to solving the food-environment-health trilemma. Although there are some examples of practice and indications of effectiveness in developing countries where fertilizer and organic resources are scarce, there has

been little academic verification of the effects of Synecoculture on soils and products. It is assumed that the fact that the biodiversity of Synecoculture field is higher than that of conventional farming can provide a positive effect on food quality and the environment, but there is little verification of these aspects in relation to human health.

In addition, to properly assess the effectiveness of Synecoculture and conduct the best possible operations, it is necessary to understand the state of the entire ecosystem, which is a complex open system. However, because of its short history, empirical knowledge has not been accumulated, and it is not expected to be easy to implement in the current socio-economic system where the success or failure of agricultural practices is measured by comparison with yields of conventional farming. In extreme cases, in conventional farming, one only needs to pay attention to the condition of a single crop, soil moisture, fertilizer, sunlight, and temperature, etc. In contrast, in Synecoculture, over 100 plant species are introduced, and the timing and spatial arrangement for these species needs to be considered, and harvesting and weed management must also be done while increasing biodiversity. This means that a vast number of variables must be taken into account, and the practitioner's cognitive ability of the entire ecosystem is required for the successful implementation of Synecoculture, which is not the case in conventional farming.

The purpose of this study is to examine the products and methods of Synecoculture in order to verify the human health-related aspects of the trilemma and the feasibility of ecosystem management of Synecoculture, and to gain knowledge and methodology that will contribute to sustainable agriculture. In order to try to achieve this purpose, this study investigated the quality of the products from a systems-level perspective, primarily by comparing the products of Synecoculture with those of conventional farming methods in relation to human health. This study also investigated the effectiveness of analytical methods based on a systems-level approach in successfully implementing "ecosystem navigation," which involves the appropriate recognition and manipulation of ecosystems.

Chapter 2 compared Synecoculture products with commercially available conventional agricultural products, and the relationship between Synecoculture and human health was discussed. Chapter 3 examined the validity of human subjective assessments and their relationship to soil analysis. Chapter 4 analyzed human intervention and ecosystem response from the perspective of ecosystem assessment and management as a complex open system, and examined methods of ecosystem navigation. Chapter 5 synthesized and discussed the results, evaluated Synecoculture, and raised future issues.

2: Comparison of Synecoculture products and conventional farming products

2-1 Introduction

Crop culture conditions impact on plant metabolic conditions [Funabashi 2015]. Previous studies have shown that the plant metabolites changes between different culture condition, namely "*in cultura*" culture conditions which are based on monoculture with external inputs and maximize individual plant growth (physiological optimum), and "*in natura*" culture conditions which are based on self-organization of ecological niche (ecological optimum) [Funabashi 2015]. Plants growing in their natural environment interact with a wide variety of other species and environments to facilitate the cycling of various nutrients and produce diverse secondary metabolites. These not only serve plant survival through physiological and allelopathic effects, but also exert health-protective functions such as anti-inflammation on the metabolism of plant consumers, including human.

In this chapter, I compared the metabolic profiles of a vegetable and tea from such a perspective between *in cultura* and *in natura* products, in relation to human health. In addition, since it is necessary for the taste of products not significantly inferior to those produced by conventional farming methods in order to be accepted by consumers, this point was also evaluated. Furthermore, I analyzed the relationship between taste and components that increase or decrease in quantity depending on the culture methods to see if human taste can pave the way for a rough estimation of certain parts of the metabolome analysis. Tea is also known to influence human energy expenditure through caffeine, catechins, and flavonoids [Stohs and Badmaev 2016]. Therefore, I observed whether the effects of drinking tea on human energy consumption differ in the two culture conditions.

Among many metabolites which influence human health, the ratio of n-6/n-3 polyunsaturated fatty acids is receiving increasing attention. Because ancient Paleolithic humans thrived on a diet of wild plants and animals rich in n-3 polyunsaturated fatty acids, it is estimated that the Paleolithic diet had a ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids of approximately 1:1 [Simopoulos 2002]. This ratio can be interpreted as the default value for the normal functioning of human metabolism. By comparison, the modern Western diet has an extremely high ratio of n-6/n-3 polyunsaturated fatty acids, which Simopoulos (2002) estimated at 15:1. This results in an increased risk of noncommunicable diseases such as cancer, cardiovascular disease, and autoimmune diseases. The fatty acid composition of grass-fed beef has a lower ratio of n-6/n-3 polyunsaturated fatty acids when compared to those produced on grain diets [Daley et al. 2010]. Similarly, in plants, the ratio of n-6/n-3 polyunsaturated fatty

379 acids has been reported to be lower in wild plants than in cultivated crops [Vardavas et
380 al. 2006]. This suggests that plant metabolism is altered in the cultivated environment
381 from its original profile in response to ecological changes. Therefore, in Section 2-2, the
382 ratios of linoleic acid (C18:2, n-6) to α-linolenic acid (C18:3, n-3) extracted from the aru-
383 gula (*Eruca sativa.* L) and Bancha grown *in cultura* and *in natura* condition were com-
384 pared.
385 In Section 2-3, I made a metabolomic comparison rather than the relatively simple
386 comparison of fatty acid ratios of Bancha (coarse green tea), made from tea plants (*Ca-*
387 *mellia sinensis.* L). I used commercially available products produced from the tea plants
388 cultivated in the same region under *in natura* culture conditions and under standard
389 *in cultura* culture conditions. Metabolomic analysis was performed, and the metabolic
390 function of the compounds that showed the largest differences between *in natura* and
391 *in cultura* culture conditions were analyzed using the chemical ontology database.
392 In Section 2-4, I conducted single-blind sensory evaluations of Bancha produced
393 in 2015 and 2017-2019 under *in cultura* and *in natura* cultivation conditions to assess the
394 taste. I also tested the feasibility of a simplified human evaluation of the products by
395 examining qualitative consistency with metabolomic analysis of the same samples
396 measured in Section 2-3.
397 In Section 2-5, I analyzed the behavior of humans who ingested the Bancha tested
398 in Section 2-4 to investigated if the two products have different effects on humans.

2-2 Fatty Acid Composition Analysis of Arugula (*Eruca sativa*)

2-2-1 Materials and Methods

Sampling

A Synecoculture farm was established on a vacant plot on the Kashiwa campus of the University of Tokyo (Kashiwa City, Chiba Prefecture, Japan) in June 2014, following the principles of Synecoculture [Funabashi 2016a] (see Appendix 1). Arugula (*Eruca sativa.* L) was grown there (sown several times from September 2014), and was used as the Synecoculture product sample, while commercially available arugula was obtained from a supermarket as the conventional sample. Both samples were thoroughly dried indoors at room temperature immediately after harvest or purchase, placed in sealable plastic bags, and stored in the dark. The cultivation was repeated 3 times, once in October-November 2014, twice in October-November 2015, and compared with the conventional samples purchased at the harvest time. The samples were labeled as SYN 2014, SYN 2015-1, SYN 2015-2 for Synecoculture samples, and CON 2014, CON 2015-1, CON 2015-2 for conventional samples.

Likewise, commercially available Bancha (coarse green tea, *Camellia sinensis*) from Synecoculture (2014 and 2015) and conventional agriculture (2015) were obtained. All Bancha samples used in this study were produced by traditional tea farmers in Watarai-cho, Mie Prefecture, Japan. From 2014 to 2015, samples were obtained from the second cutting (leaves below the shoots) of the first harvest, which takes place annually in late May to early June, were used. Standard steaming, rubbing, and drying processes were performed at a local machine factory. During this process, leaves from more than 7000 m^2 of cultivated land within a radius of 2 km were blended in order to average out plot-specific variations. Conventional tea was produced under conventional monoculture culture conditions (referred as *in cultura* culture condition), which follows the standard protocol of the Ise branch of the agricultural cooperative and routinely uses synthetic and organic fertilizers, pesticides, fungicides, and herbicides. Synecoculture tea was produced under mixed dense polyculture conditions without tillage, fertilizers, or agrochemicals (referred as *in natura* culture condition). These are referred to as SYN 2014 Tea, SYN 2015 Tea, and CONV 2015 Tea, respectively.

Fatty acid extraction

The process from extraction to analysis was based on the method of Takeshita et al (2014). Leaves were taken from a dried sample, 60 mg of each grinded in a mortar, and collected in a tempered glass tube (15 mLTST-SCR 16-100, IWAKI). In addition, 1.5mL methanol (Wako special grade) and 5mL MTBE (t-butyl methyl ether (Wako first

grade)) were added in this order using a glass pipette. After each addition, the mixture was thoroughly mixed using a vortex mixer, and an ultrasonic cell disruption machine (5203FZT, Ohtake Seisakusho) twice for 30 seconds (with an interval of about 8 seconds) to ensure efficient penetration of the solvent. The glass tube was ice-cooled during the interval to avoid excessive heating of the sample. The samples were shaken at 150 times/min for 2 hours at room temperature using a shaking incubator (BIO-SHAKER BR-40LF, TAITEC), and centrifuged (2,000 rpm) to separate insoluble materials. The solvent was collected in an organic solvent-resistant syringe (2-4031-03, AS ONE), filtered through a PTFE filtration filter (hole diameter 0.45 μm, Millex: SLLHH04NL) attached to the syringe, and collected in a 30 mL vial (1-3501-06, AS ONE). As an internal standard, 0.5 mL of a 19-carbon nonadecanoic acid solution (72332-1G-F SIGMA ALDRICH NEAT≥99.5% (GC) CAS 646-30-0 MW 298.50 g/mol) was added. They were then allowed to dry overnight at 50°C in a nitrogen environment using an oven.

To the dried samples, 1 mL of 3N-MeOH HCl (33355 SUPELCO, SIGMA ALDRICH) was added and incubated at 85°C for 2.5 hours using a water bath, keeping the lid closed. The mixture was cooled to room temperature. Further, 0.5 mL of water (DW) and 1 mL hexane (n-hexane, Wako special grade) were added in this order, stirred thoroughly, and the upper hexane layer was collected with a Pasteur pipette. This procedure of adding 1 mL of hexane and collecting the upper hexane layer was repeated three times, yielding 3 mL of the extract to be analyzed.

GC-MS analysis

The extracted samples were analyzed using a gas chromatography mass spectrometer (QP-2010Plus, Shimadzu Corporation) with a hydrogen flame ionization detector. The column used was SUPELCO SP-2380 (30 m × 0.25 mm × 0.20 μmm, SIGMA ALDRICH). Analytical conditions were as follows: Sample injection was set 1 μL, carrier gas (helium) 24.2 cm/s, make-up gas (helium) 20 mL/min, injector and detector temperature 250°C, and oven temperature set to 140°C for 1 minute, then 4°C/min up to 220°C.

Fatty acids were identified by sample retention time and mass spectrum from data of the fatty acid standard mix (Supelco 37-component FAME mix standard, Supelco) under the same analytical conditions.

In the chromatograms obtained (Appendix 2), 10 peaks were extracted in descending order of area, and peaks with an area ratio of 1% or less and peaks of the internal standard (C19) were excluded. The total area of all peaks was normalized based

on the area of the internal standard peak (the areas of the other peaks were divided by the area of the internal standard). The ratio of individual peaks to the total area of the remaining peaks was also calculated in percentage.

The percentage of linoleic acid (C18:2) peak area was compared to the percentage of linolenic acid (C18:3) peak area. Since linoleic acid (C18:2) has geometric isomers and two peaks were detected in this analysis, the sum of the two peaks was used as the peak area of linoleic acid (C18:2). The extraction of the same samples was performed again 10 months later with SYN 2014 arugula to examine the effects of long-term storage, and labeled as SYN 2014 A. Student's t-tests (one-sided) were performed between SYN 2014, 2015-1, 2015-2 and CON 2014, 2015-1, 2015-2 for area of linoleic acid, α-linolenic acid, and n-6/n-3 ratio.

2-2-2 Results

Averaged area of linoleic acid (C18:2) and α-linolenic acid (C18:3) peaks across all the sampling times were 40.7±4.66% and 33.2±1.91% in the naturally grown arugula and 59.2±6.97% and 18.5±4.71% in the conventional products, respectively (Table 1a). The concentration of α-linolenic acid was higher ($p=0.026$), and the ratio of n-6/n-3 was lower ($p=0.016$) in the naturally grown arugula compared to those in the conventional products.

Results of SYN 2014 showed that the peak area of linoleic acid and α-linolenic acid were lost during the storage period with a decrease of 15.3% and 31.8%, respectively. However, the n-6/n-3 ratio after 10 months of storage was still lower than that of conventionally grown arugula.

The same trend as for arugula was observed for Bancha, with a lower n-6/n-3ratio in the Synecoculture sample (Table 1b).

Table 1a. Summary of GC-MS results with arugura.
LA represents linoleic acid, ALA represents α-linolenic acid, and n-6/n-3 ratio represents the area of linoleic acid divided by the area of α-linolenic acid, respectively.

ID	SYN 2014	SYN 2014A	SYN 2015-1	SYN 2015-2	CON 2014	CON 2015-1	CON 2015-2
Farming Method	Synecoculture	Synecoculture	Synecoculture	Synecoculture	Conventional	Conventional	Conventional
LA area	12.4	10.5	9.6	8.7	24.5	9.5	13.7
ALA area	8.5	5.8	9.2	7.2	7.0	4.4	3.1

Total fatty acids area	27.2	21.6	26.3	21.7	38.6	18.6	21.9
LA area ratio (%)	45.6	48.6	36.4	40.0	63.6	51.2	62.8
ALA area ratio (%)	31.2	26.8	35.0	33.3	18.0	23.5	14.1
ALA/LA (%)	68.4	55.1	96.3	83.3	28.3	45.9	22.4
n-6/n-3 ratio	1.46	1.81	1.04	1.20	3.53	2.18	4.46

Table 1b. Summary of GC-MS results with Bancha.

The description follows Table 1a.

ID	SYN2014 Tea	SYN2015 Tea	CON2015 Tea
Farming Method	Synecoculture	Synecoculture	Conventional
LA area	9.0	10.1	26.3
ALA area	5.5	5.0	4.5
Total fatty acids area	17.6	17.2	32.1
LA area ratio (%)	51.5	58.7	81.8
ALA area ratio (%)	31.6	29.0	13.9
ALA/LA (%)	61.4	49.3	17.0
n-6/n-3 ratio	1.63	2.03	5.88

2-2-3 Discussion

One of the objectives of this study was to test whether the fatty acid composition could differ between the two culture conditions, and the results suggested that the Synecoculture product can be potentially different from the conventionally grown product.

Since there is a difference of storage time between the harvest and extraction of Synecoculture and conventional samples for arugula (it takes several days for the conventional samples to be transported from the farm to the store), the Synecoculture samples were tested after long-term storage to see if the days could cause a significant decrease in unsaturated fatty acids, which turned out to be limited. In general, unsaturated fatty acids are easily oxidized, while α-linolenic acid is not easily oxidized in chloroplast glycolipids [Yamaguchi et al. 2012]. This is consistent with the results of this study, in which the amount of α-linolenic acid in Synecoculture arugula remained stable during a 10-month storage period.

Due to the small sample size, differences in soil properties, harvest timing, and storage methods etc., it is difficult to draw the conclusion that this difference in fatty

520 acid composition occurs between *in natura* and *in cultura* products, but I would like to
521 proceed the following discussions.
522 It is reasonable to assume that the lower amount of n-3 fatty acids in commercial
523 arugula than in Synecoculture products was due to artificial manipulation in conven-
524 tional farming. Of the three operations of conventional farming, fertilizer appears to be
525 the one most directly involved in the physiological state of the plant. The physiological
526 state of the crop is likely to be altered in some way by fertilizers and other factors that
527 are not present under normal natural conditions, resulting in faster growth than in na-
528 ture. In fact, it has been reported that fertilizer use reduces the percentage of linolenic
529 acid to total oil in *Nigella sativa* seed [Moradzadeh et al. 2021]. This is supported by the
530 fact that a similar trend was observed with Bancha in this experiment, although the
531 plant species were different.
532 Both Synecoculture and conventional arugula n-6/n-3 ratios in this study were
533 within the recommended ratio. However, in a situation where the overall n-6/n-3 ratio
534 is increasing as a result of continued Western-style diets, the consumption of Syneco-
535 culture, leafy vegetables, which has a low n-6/n-3 ratio, may be beneficial in maintain-
536 ing human health by contributing to reducing the risk of chronic diseases. Actually,
537 leafy vegetables are considered an important source of α-linolenic acid [Pereira et al.
538 2001].
539 Next, I will discuss the results of the metabolome analysis of coarse green tea,
540 since a similar trend was observed in tea plants as in arugula.
541
542

2-3 Metabolome Analysis of Bancha (coarse green tea)

2-3-1 Materials and Methods

Same as the Section 2-2-1, all Bancha samples used in this study were produced by traditional tea farmers in Watarai-cho, Mie Prefecture, Japan. From 2014 to 2019.

In total, 11 sets of samples were prepared, consisting of dried coarse green tea from the Synecoculture fields collected annually between 2014 to 2019 and conventional tea culture between 2015 to 2019. I hereafter call these samples as Syneco 2014, Syneco 2015, Syneco 2016, Syneco 2017, Syneco 2018, Syneco 2019, Conv 2015, Conv 2016, Conv 2017, Conv 2018 and Conv 2019, respectively.

2-3-1-1 Metabolome Analysis

2-3-1-1-1 Metabolite Extraction

Samples were made in tea bags of 3.0 g each, boiled in 1l of ultrapure water (Milli-Q) at 90–93℃ for 10 min in a glass beaker and left at room temperature for 2 h. Each of these samples was extracted for metabolome analyses: The extraction protocol was slightly different between 2014-2017 samples and 2018-2019 samples with the updating of the analysis equipment. For 2014–2017 samples: Each 100 μL sample was mixed with 300 μL methanol and centrifuged with 10,000× g, 10min, 4 °C. The supernatant was filtered with PTFE filter (Millipore, Cat.SLLGH04NK) and centrifuged through Monospin C18 spin columns with 5000× g, 2min, 4 °C in order to remove insoluble matters and low polarity components. A mock sample of ultrapure water was prepared with the same procedure, and was used to evaluate and remove background noise contained in the sample preparation and/or LC-MS analysis.

For 2018–2019 samples: Each 100 μL sample was mixed with 300 μL methanol and centrifuged with 15,000 rpm, 10min. As pretreatment for column equilibration, 100% methanol centrifuged through Monospin C18 spin columns with 5000×g, 2 min, then, 75% methanol centrifuged through the same columns with 5000×g, 2 min. Then, the supernatant of the sample was centrifuged through the same columns with 5000×g, 2 min. After that, the supernatant of the sample was filtered with a 0.2-μm filter. A mock sample of ultrapure water was prepared with the same procedure, and was used to evaluate and remove background noise contained in the sample preparation and/or LC-MS analysis.

2-3-1-1-2 LC-MS Analysis of 2014–2017 Samples

LC−MS analysis was performed with a combination of Agilent 1200 series (Agilent) and Thermo fisher scientific LTQ ORBITRAP XL (Thermo Fisher Scientific (A)). The parameters of measurement are summarized in Appendix 3.

After converting raw data (obtained from LTQ ORBITRAP XL) to a text file with the use of ProteoWizard [Kessner et al. 2008], LC-MS data were analyzed using PowerGet ver. 3.5.7 (KOMICS (A)) with the following procedure to attribute each MS peak to a chemical formula:

1. Empirical detection of compound peaks, calculation of accurate mass, calculation of compound peak intensity.
2. Differentiation of simultaneous elution peaks with respect to the profile of adduct ion peaks, ionization mode, and natural 13C isotopic compound peaks.
3. Matching between MS peaks and MS/MS data, calculation of 13C/12C isotope ratio with ion intensity in order to estimate C number in each compound, and estimation of ionization mode.
4. Aggregation and sorting of compound peaks with respect to the elution time, accurate mass, and MS/MS patterns for all samples.
5. Matching of calculated mean accurate mass with monoisotopic compounds in public databases [KEGG][Flavonoid Viewer] with the use of MF Searcher [Sakurai et al. 2013] and derivation of a corresponding chemical formula.
6. Truncate the compound peaks with less than 2 times intensity of the mock sample.

The parameters of these analyses are summarized in Appendix 3.

2-3-1-1-3 LC-MS Analysis of 2018–2019 Samples

LC−MS analysis was performed with a combination of Ultimate 3000 RSLC (Thermo Fisher Scientific (B)) and Q Exactive (Thermo Fisher Scientific) (Q Exactive). Samples were analyzed 3 times for each sample.

After converting raw data (obtained from Q Exactive) to a text file with the use of ProteoWizard, LC-MS data were analyzed using PowerGetBatch (KOMICS (B)) with the following procedure to attribute each MS peak to a chemical formula:

1. Empirical detection of compound peaks, calculation of accurate mass, calculation of compound peak intensity
2. Ionization status judgment
3. Alignment of compound peaks

4. Matching of calculated mean accurate mass with monoisotopic compounds in public database with the use of MF Searcher and derivation of a corresponding chemical formula

The parameters of these analyses are summarized in Appendix 3.

As a premise, this metabolome analysis is a result of projecting measured exact masses onto the public database, so there is not sufficient resolution for the accurate distinction between structural isomers.

2-3-1-1-4 Integration of Metabolite Data of 2014–2019 Samples

Based on the exact mass detected, MF Searcher [Sakurai et al. 2013] was used to match the same compound of each year with an error of 1 ppm in KEGG database, and integrated the metabolomic data of all years (See Appendix 4). The same procedure was also performed in Flavonoid Viewer [Flavonoid Viewer] to compare the total estimated amount of flavonoids (see Appendix 5). When multiple structural isomers were detected from the KEGG database, the intensity was taken as the average value for each detected year, because these cannot be distinguished at the present resolution of LC-MS.

2-3-1-1-5 Biological and Technical Replicate

The sample of tea leaves were obtained from the mixed harvest over entire fields, at a total of 3 times mixing (triple homogenization) at the time of harvesting, kneading, and drying, so it was not possible to sample each small area or individual tea tree as biological replicates. Therefore, this was regarded as a representative of the entire field for each year, and biological variance was greatly averaged through harvesting and processing in this study. Since the tea leaves were inevitably homogenized through the processing, it was not possible to take plant-wise or area-wise biological replicates for the product analyzed.

LC-MS analysis was performed once for the 2014–2017 and three times for the 2018–2019 samples. To assess the homogeneity of the tea products and reproducibility of measurement, the 2014–2019 samples were extracted three times with the same procedure of sample extraction as “technical replicate”, and the temporal changes in absorbance of the samples were measured with a spectrophotometer (U-2010, HITACHI). There was almost no difference in the extraction and no change after a certain period of time, 6 h (1/40–1/10000 error of the absorbance). The summary of the sample replicates and LC-MS analysis method are shown in Table 2.

Table 2. Summary of the sample replicates and LC-MS analysis method.

Measurement		Parameters of each Sampling Year					
Replicate	**Method**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**
Technical replicate	Photospectrometry (absorbance error)	3 (1/100–1/10000)	3 (1/100–1/10000)	3 (1/40–1/1000)	3 (1/50–1/1000)	3 (1/40–1/10000)	3 (1/100–1/10000)
	LC-MS (intensity error)	1 (estimated CV: 10–20%)	1 (estimated CV: 10–20%)	1 (estimated CV: 10–20%)	1 (estimated CV: 10–20%)	3 (measured CV: 16.2%)	3 (measured CV: 16.8%)
HPLC		Agilent 1200 series				Ultimate 3000 RSLC	
Tea Sampling and Processing		Same protocol					

2-3-1-2 Statistical Analysis

For each chemical formula obtained, the mean value of the intensity for each year was calculated for Syneco and Conv, respectively, and normality was tested with the Shapiro-Wilk test. Also, the Welch's t-test (applicable even if the normally distributed data do not have homoscedasticity) and the Brunner-Munzel test (assumptions for the homogeneity of variance and normality are not needed) [Brunner and Munzel 2000] were performed with Syneco 2014–2019 and Conv 2015–2019 for the LC-MS raw and logarithmic intensity values of the identified compounds, using 6 data from Syneco 2014-2019 and 5 data from Conv 2015-2019. For the 2018 and 2019 samples, the mean values of the three measurements on the same samples were used. Welch's t-test was performed with Microsoft Excel ver. 16.16.21, and a Brunner–Munzel test was performed with statistical analysis software R ver. 3.5.0.

The variance value representing the magnitude of the year-to-year variation of each compound was calculated, and the distribution of the variances was compared by F-test between Syneco and Conv samples. For the 2018 and 2019 samples, the mean values of the three measurements on the same samples were used. F-test was performed with Microsoft Excel ver. 16.16.21.

In order to investigate the overall effects of culture conditions on the metabolic state, the principal component analysis (PCA) was used after the normalization of the intensity values for each compound. PCA was applied repeatedly by increasing the number of compounds having a high positive/negative eigenvector to a PC to see the separability of Syneco and Conv samples on the PC plane (hereafter denoted as LS-PCA, meaning linear separation with PCA). The compounds that were sufficient to separate the two culture conditions are called "distinctive loadings" for convenience. The LS-PCA was performed by a statistical analysis software R ver. 3.5.0.

These analyses were performed with 2014–2019 samples, along with the subsets of 2014–2017 and 2018–2019 samples, because LC-MS parameters are different. I call these groupings as three PCA groups 2014–2017, 2018–2019, and 2014–2019. See the PCA plot of 2014–2017 and 2018–2019 samples in Appendix 6 and 7 for the complete result.

All structural isomers of the 130 distinctive loadings on KEGG were projected onto the "map01110 Biosynthesis of secondary metabolites" of KEGG PATHWAY (see Appendix 8). In order to see the distribution of chemical formulas expressed only in Syneco and Conv, respectively, and those expressed in common, visualization of the distribution was performed (See the Appendix 9).

2-3-1-3 Metabolome Categorization

The list of 130 chemical formulae obtained with LS-PCA was projected to KEGG (Kyoto Encyclopedia of Genes and Genomes) [Kanehisa and Goto 2000] databases to annotate possible physiological functions. KEGG API was used to mine the KEGG BRITE database and KEGG PATHWAY in order to categorize the compounds according to the functional classification. Each chemical formula was attributed to the hierarchical ontology of these databases including the matching with structural isomers, as an extensive interpretation of obtained metabolome data on known physiological functions.

Welch's t-test and Brunner-Munzel test were performed in each category of KEGG BRITE and KEGG PATHWAY, with Syneco 2014–2019 and Conv 2015–2019 compounds intensity and logarithmic intensity, in order to investigate compound category-wise intensity differences between Syneco and Conv. The mean values of the intensity of the same chemical formula in same category, and each value of the actual measured intensity in same category were used for each test. This is in addition to the t-test and Brunner-Munzel test to see if there is a difference between Syneco and Conv when grouped together in the same category, instead of each single chemical formula.

2-3-2 Results

2-3-2-1 Metabolome Analysis

Exact mass and intensity data of 1055, 815, 714, 1080, and 843 compound peaks were obtained in the Syneco and Conv samples of the years 2015 (including 2014, in same LC-MS analysis), 2016, 2017, 2018, and 2019, respectively.

2-3-2-2 Statistical Analysis

Using the MF searcher, the exact masses of 342 compound peaks were matched with known compounds in the KEGG database (Appendix 4). Among them, the average raw intensity of each 125 compounds was greater for Syneco samples than Conv samples, and 217 compounds for Conv samples than Syneco samples. The Shapiro-Wilk test rejected the normality of the set of means per chemical formula for both Syneco and Conv. The result of Welch's *t*-test for the raw intensity showed that only one compound (C19H32O8) was significantly different with a 5% significance level (p = 0.0143), but the others were not significant. The result of the Brunner-Munzel test for the raw intensity showed that only four compounds (C33H40O21, C19H32O8, C5H11N3O2, C16H18O8) were significantly different with a 5% significance level (p = 0.0123, 0.00852, 0.0161, 0.0336, respectively), but the others were not significant.

The result of Welch's *t*-test for the logarithmic intensity showed that only one compound (C5H11N3O2) was significantly different with a 5% significance level (p = 0.0166), but the others were not significant. The result of the Brunner–Munzel test for the logarithmic intensity showed that only 4 compounds (C33H40O21, C19H32O8, C5H11N3O2, C16H18O8; the same as for the raw intensity) were significantly different with a 5% significance level (p = 0.0123, 0.00852, 0.0161, 0.0336, respectively), but the others were not significant. C33H40O21 and C19H32O8 had higher intensities in Syneco, while C5H11N3O2 and C16H18O8 had higher intensities in Conv.

The results of F-test between the yearly variances of the samples for each of the 342 compounds showed that 58 compounds were significantly different between the Syneco and Conv samples with the significance level of 5%. In terms of the variance values representing the yearly fluctuation for each compound, 132 compounds in Syneco were greater than Conv, and 210 compounds in Conv were greater than Syneco. The F-test comparing the variances between Syneco and Conv for the intensity distribution of 342 compounds for all years of sampling showed a statistically significant difference ($p = 7.84 \times 10^{-49}$), with a higher variance of Conv. Welch's two-sided *t*-test was also performed on the difference between the mean values of the variances, but there was no significant difference (p = 0.651). These statistical analyses were summarized in Appendix 9.

The result of PCA in Figure 3 revealed that PC3 could linearly separate Syneco and Conv completely (horizontal axis in Figure 3b). PC1 seemed to represent the differences between the two LC-MS analysis conditions that changed between 2014–2017 and 2018–2019 samples (Figure 3a). PC2 mainly represented the yearly fluctuation of all samples that showed three distinctive clusters of 2014–2017, 2018, and 2019 (refer to

Appendix 6 for the importance and cumulative proportion of the components, and Appendix 7 for other PC plots within the subsets 2014–2017 and 2018–2019). In other words, the errors that can occur in my method were aggregated in PC1 and PC2, and PC3 was considered to represent a robust characteristic that separates Syneco and Conv samples.

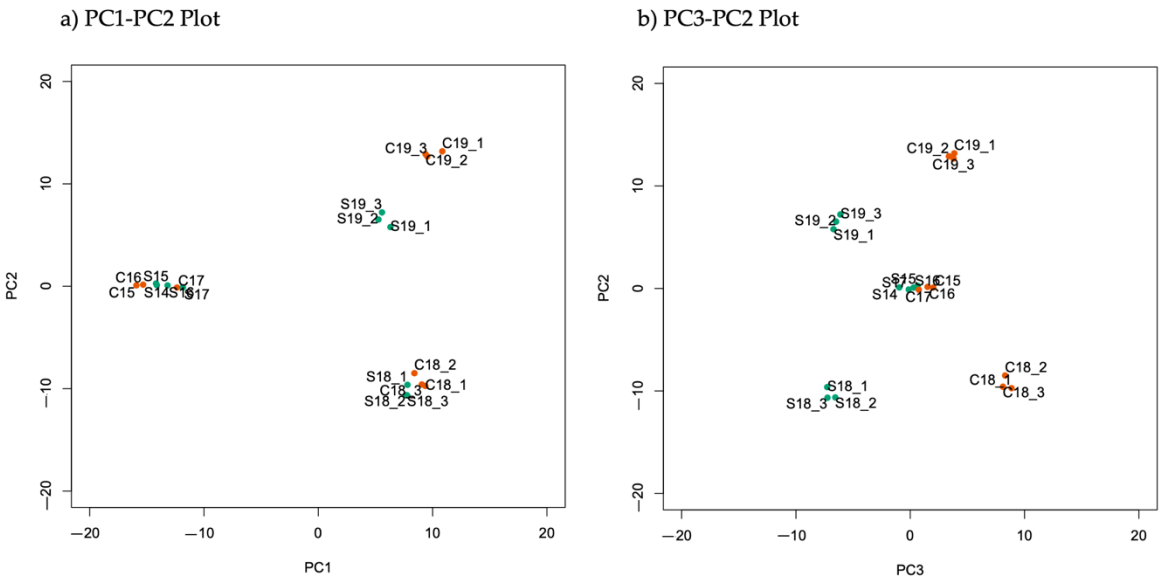


Figure 3. Principal component analysis (PCA) plot based on the intensity of the compounds in coarse green tea samples.
(**a**) PCA plot of PC1-PC2. (**b**)PCA plot of PC2-PC3. S14, S15, S16, S17, S18, S19, C15, C16, C17, C18, and C19 correspond to the samples Syneco 2014, Syneco 2015, Syneco 2016, Syneco 2017, Syneco 2018, Syneco 2019, Conv 2015, Conv 2016, Conv 2017, Conv 2018, and Conv 2019, respectively. The 2014–2017 samples are based on a single measurement, while the 2018 and 2019 samples consist of 3 different measurements of intensity data.

As shown in Figure 4, the Syneco and Conv samples were completely separated with LS-PCA using the top 65 compounds of PC3 negative/positive loadings (in total 130 compounds). Hereafter I call these top 130 negative and positive loading parameters as Syneco and Conv-distinctive parameters, respectively. I used these 130 distinctive compounds for the ontological categorization using KEGG BRITE and KEGG PATHWAY databases.

Using the MF searcher, 97 flavonoid compounds were listed by matching the metabolome data to the Flavonoid Viewer (Appendix 5). The total intensity of the

detected flavonoids in all 2014–2019 samples were compared between Syneco and Conv samples. Although Conv samples tended to contain more flavonoids, it showed no statistically significant difference ($p = 0.786$, two-sided Welch's t-test) in total intensity of flavonoids.

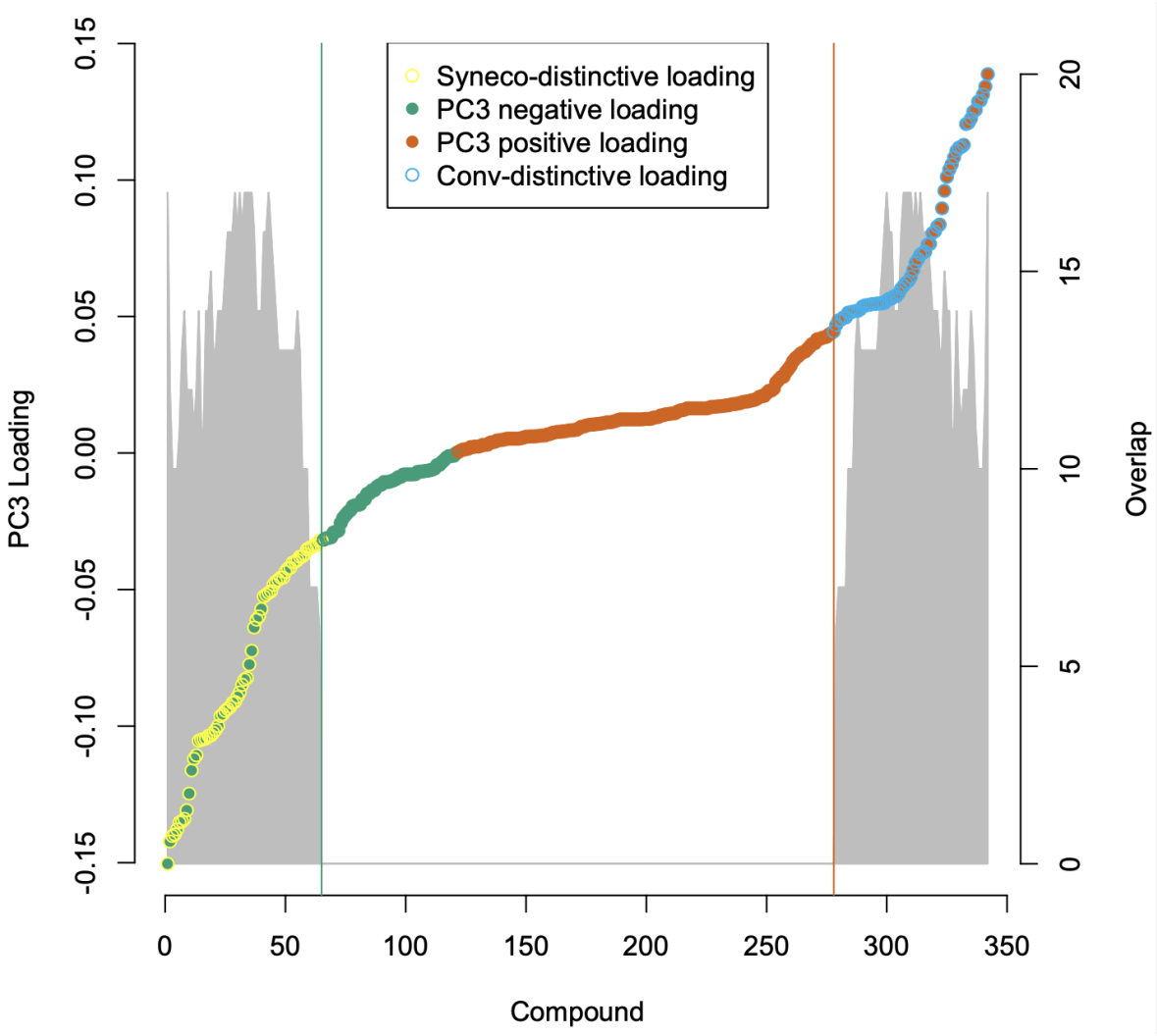


Figure 4. Syneco/Conv distinctive-loadings identified by linear separation with PCA (LS-PCA) and PC3 loading plot of the intensity of the compounds in coarse green tea samples.

For negative and positive loadings of PC3 (left Y-axis) aligned in ascending order (X-axis), the 65 smallest and 65 largest loadings of the compounds are separated by 2 vertical lines (green and orange, respectively). The overlap numbers (top edge of the gray area) represent the degree of separation between Syneco and Conv when LS-PCA was performed. It is given by 19-

(#Syneco>maximum(CONV)+#Syneco<minimum(Conv)+#Conv>maximum(Syneco)+#Conv<minimum(Conv)). In case of complete separation, this will be 0. When PCA was performed by increasing the number of upper loadings by one from each side, the Overlap became 0 for the first time when PCA was performed with 65 loadings from each side, and remained 0 thereafter. Hereafter I call these 65 negative and positive loadings "Syneco-distinctive loadings" and "Conv-distinctive loadings" which are plotted with yellow and light blue hollow circles, respectively.

2-3-2-3 Metabolome Categorization

I projected the top 130 (65 positive and 65 negative) of PC3 loadings to KEGG BRITE (Table 2) and KEGG PATHWAY (Table 4), and looked to the KEGG BRITE compound classification. The negative PC3 loadings that characterized Syneco samples expressed more diversity of allelochemicals than Conv samples such as phytochemicals, alkaloids, phenylpropanoids, and steroids (Table 3). The "Phytochemicals" include the subcategories of alkaloids, flavonoids, phenylpropanoids, shikimate/acetate-malonate pathway derived compounds, terpenoids, polyketides, fatty acids related compounds, amino acid related compounds, and others, according to the notation in KEGG BRITE database. KEGG PATHWAY results showed that Conv had more formulas in the category of primary metabolites such as Carbohydrate metabolism, Nucleoside metabolism and Amino acid metabolism (Table 4 page 1).

Table 3. KEGG BRITE compound classification.

The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG BRITE were shown. Uncertainty Score is the sum of inverse numbers of structural isomers for each chemical formula. The smaller the number, the greater the number of structural isomers, indicating the higher uncertainty of compound identification. See Appendix 10, 11, and 12 for more details.

	Syneco		**Conv**	
	# Formulae	**Uncertainty Score**	**# Formulae**	**Uncertainty Score**
Flavonoid	10	4.075	13	6.751587
Phytochemical	41	15.98387	25	12.61299
Alkaloid	4	3.125	3	1.47619
Phenylpropanoid	17	3.904167	4	1.821429
Steroid	2	1.083333	0	-
Total	74	28.17137	45	22.662196

Table 4. KEGG PATHWAY compound classification. (page 1)
The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG PATHWAY hierarchy were shown.

Culture Condition			Syneco		Conv	
Category			#Formulae	Uncertainty Score	#Formulae	Uncertainty Score
All Categories			152	39.32423687	226	56.47841953
1. Metabolism			147	38.19923687	168	42.42704868
1.0 Global and overview maps			57	15.5296398	56	17.70079175
	map01100	Metabolic pathways	18	5.601724664	20	8.437734488
	map01110	Biosynthesis of secondary metabolites	21	5.025534188	17	5.276477178
	map01120	Microbial metabolism in diverse environments	9	2.84702381	5	1.382539683
	map01200	Carbon metabolism	1	1	1	0.071428571
	map01210	2-Oxocarboxylic acid metabolism	2	0.333333333	6	1.166305916
	map01230	Biosynthesis of amino acids	1	0.166666667	6	1.166305916
	map01220	Degradation of aromatic compounds	5	0.555357143	1	0.2
1.1 Carbohydrate metabolism			0	0	4	0.285714286
	map00020	Citrate cycle (TCA cycle)	0	0	1	0.071428571
	map00040	Pentose and glucuronate interconversions	0	0	1	0.071428571
	map00053	Ascorbate and aldarate metabolism	0	0	1	0.071428571
	map00630	Glyoxylate and dicarboxylate metabolism	0	0	1	0.071428571
1.2 Energy metabolism			2	1.125	2	0.182539683
	map00720	Carbon fixation pathways in prokaryotes	1	1	1	0.071428571
	map00680	Methane metabolism	1	0.125	1	0.111111111
1.3 Lipid metabolism			2	1.5	1	0.2
	map00061	Fatty acid biosynthesis	1	0.5	0	0
	map00073	Cutin, suberine and wax biosynthesis	0	0	1	0.2
	map00140	Steroid hormone biosynthesis	1	1	0	0
1.4 Nucleotide metabolism			0	0	2	0.666666667
	map00230	Purine metabolism	0	0	2	0.666666667
1.5 Amino acid metabolism			11	1.476190476	18	6.015151515
	map00250	Alanine, aspartate and glutamate metabolism	0	0	1	0.071428571
	map00260	Glycine, serine and threonine metabolism	0	0	1	0.142857143
	map00270	Cysteine and methionine metabolism	0	0	1	1
	map00310	Lysine degradation	0	0	1	1
	map00220	Arginine biosynthesis	0	0	2	0.75
	map00330	Arginine and proline metabolism	0	0	2	1.25
	map00350	Tyrosine metabolism	5	0.580357143	2	0.611111111
	map00360	Phenylalanine metabolism	3	0.3125	2	0.202020202
	map00380	Tryptophan metabolism	1	0.25	3	0.642857143
	map00400	Phenylalanine, tyrosine and tryptophan biosynthesis	2	0.333333333	3	0.344877345
1.6 Metabolism of other amino acids			0	0	4	1.702020202

Table 4. KEGG PATHWAY compound classification. (page 2)
The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG PATHWAY hierarchy were shown. Due to the large size of the table, there is a duplication with the previous page.

Culture Condition		Syneco		Conv	
Category		#Formulae	Uncertainty Score	#Formulae	Uncertainty Score
All Categories		152	39.32423687	226	56.47841953
1. Metabolism		147	38.19923687	168	42.42704868
1.6 Metabolism of other amino acids		0	0	4	1.702020202
map00410	beta-Alanine metabolism	0	0	1	1
map00440	Phosphonate and phosphinate metabolism	0	0	1	0.5
map00460	Cyanoamino acid metabolism	0	0	2	0.202020202
1.8 Metabolism of cofactors and vitamins		4	2.666666667	3	1.222222222
map00730	Thiamine metabolism	0	0	1	0.111111111
map00770	Pantothenate and CoA biosynthesis	0	0	1	1
map00785	Lipoic acid metabolism	1	0.5	0	0
map00790	Folate biosynthesis	1	1	0	0
map00670	One carbon pool by folate	1	1	0	0
map00130	Ubiquinone and other terpenoid-quinone biosynthesis	1	0.166666667	1	0.111111111
1.9 Metabolism of terpenoids and polyketides		8	1.825213675	12	3.258363712
map00900	Terpenoid backbone biosynthesis	0	0	1	0.052631579
map00902	Monoterpenoid biosynthesis	2	1.076923077	0	0
map00909	Sesquiterpenoid and triterpenoid biosynthesis	1	0.011111111	2	0.14354067
map00904	Diterpenoid biosynthesis	0	0	1	0.125
map00981	Insect hormone biosynthesis	1	0.166666667	2	0.14354067
map00908	Zeatin biosynthesis	0	0	1	1
map00903	Limonene and pinene degradation	1	0.076923077	0	0
map00281	Geraniol degradation	1	0.076923077	0	0
map01059	Biosynthesis of enediyne antibiotics	1	0.25	1	0.111111111
map01057	Biosynthesis of type II polyketide products	0	0	2	1.5
map01053	Biosynthesis of siderophore group nonribosomal peptides	0	0	1	0.071428571
map01055	Biosynthesis of vancomycin group antibiotics	1	0.166666667	1	0.111111111
1.10 Biosynthesis of other secondary metabolites		33	9.054700855	36	6.420779221
map00232	Caffeine metabolism	1	1	0	0
map00333	Prodigiosin biosynthesis	1	0.076923077	0	0
map00940	Phenylpropanoid biosynthesis	8	1.2875	4	0.785353535
map00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0	0	2	0.583333333
map00941	Flavonoid biosynthesis	5	1.625	6	1.242063492
map00944	Flavone and flavonol biosynthesis	5	2.45	3	0.485714286
map00942	Anthocyanin biosynthesis	0	0	1	0.5
map00943	Isoflavonoid biosynthesis	1	0.125	3	0.325396825
map00901	Indole alkaloid biosynthesis	0	0	1	0.142857143
map00950	Isoquinoline alkaloid biosynthesis	3	0.354166667	1	0.111111111
	Tropane, piperidine and pyridine				

Table 4. KEGG PATHWAY compound classification. (page 3)

The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG PATHWAY hierarchy were shown. Due to the large size of the table, there is a duplication with the previous page.

Culture Condition		Syneco		Conv	
Category		#Formulae	Uncertainty Score	#Formulae	Uncertainty Score
All Categories		152	39.32423687	226	56.47841953
1. Metabolism		147	38.19923687	168	42.42704868
1.10 Biosynthesis of other secondary metabolites		33	9.054700855	36	6.420779221
map00232	Caffeine metabolism	1	1	0	0
map00333	Prodigiosin biosynthesis	1	0.076923077	0	0
map00940	Phenylpropanoid biosynthesis	8	1.2875	4	0.785353535
map00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0	0	2	0.583333333
map00941	Flavonoid biosynthesis	5	1.625	6	1.242063492
map00944	Flavone and flavonol biosynthesis	5	2.45	3	0.485714286
map00942	Anthocyanin biosynthesis	0	0	1	0.5
map00943	Isoflavonoid biosynthesis	1	0.125	3	0.325396825
map00901	Indole alkaloid biosynthesis	0	0	1	0.142857143
map00950	Isoquinoline alkaloid biosynthesis	3	0.354166667	1	0.111111111
map00960	Tropane, piperidine and pyridine alkaloid biosynthesis	0	0	1	0.090909091
map00232	Caffeine metabolism	1	1	0	0
map00965	Betalain biosynthesis	0	0	1	0.111111111
map00966	Glucosinolate biosynthesis	0	0	3	0.344877345
map00332	Carbapenem biosynthesis	0	0	1	0.25
map00261	Monobactam biosynthesis	1	0.166666667	1	0.111111111
map00401	Novobiocin biosynthesis	2	0.416666667	1	0.111111111
map00404	Staurosporine biosynthesis	0	0	1	0.142857143
map00999	Biosynthesis of various secondary metabolites - part 1	1	0.011111111	0	0
map00998	Biosynthesis of various secondary metabolites - part 2	4	0.541666667	5	1.011544012
map00997	Biosynthesis of various secondary metabolites - part 3	0	0	1	0.071428571
1.11 Xenobiotics biodegradation and metabolism		14	1.802380952	7	1.520634921
map00627	Aminobenzoate degradation	0	0	1	0.5
map00623	Toluene degradation	1	0.0625	0	0
map00622	Xylene degradation	3	0.305357143	0	0
map00633	Nitrotoluene degradation	1	0.166666667	0	0
map00642	Ethylbenzene degradation	2	0.205357143	0	0
map00643	Styrene degradation	1	0.0625	0	0
map00363	Bisphenol degradation	1	0.0625	0	0
map00626	Naphthalene degradation	3	0.354166667	1	0.2
map00624	Polycyclic aromatic hydrocarbon degradation	1	0.083333333	2	0.311111111
map00980	Metabolism of xenobiotics by cytochrome P450	0	0	2	0.342857143
map00982	Drug metabolism - cytochrome P450	1	0.5	1	0.166666667
1.12 Chemical structure transformation maps		16	3.219444441	23	3.252164502
map01060	Biosynthesis of plant secondary metabolites	4	0.854166667	5	1.416305916
map01061	Biosynthesis of phenylpropanoids	6	1.416666667	6	0.737734488

Table 4. KEGG PATHWAY compound classification. (page 4)

The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG PATHWAY hierarchy were shown. Due to the large size of the table, there is a duplication with the previous page.

Culture Condition		Syneco		Conv	
Category		#Formulae	Uncertainty Score	#Formulae	Uncertainty Score
All Categories		152	39.32423687	226	56.47841953
1. Metabolism		147	38.19923687	168	42.42704868
1.12 Chemical structure transformation maps		16	3.219444441	23	3.252164502
map01060	Biosynthesis of plant secondary metabolites	4	0.854166667	5	1.416305916
map01061	Biosynthesis of phenylpropanoids	6	1.416666667	6	0.737734488
map01062	Biosynthesis of terpenoids and steroids	1	0.011111111	1	0.071428571
map01063	Biosynthesis of alkaloids derived from shikimate pathway	3	0.354166667	4	0.416305916
map01064	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	1	0.08333333	2	0.162337662
map01065	Biosynthesis of alkaloids derived from histidine and purine	0	0	1	0.071428571
map01066	Biosynthesis of alkaloids derived from terpenoid and polyketide	1	0.5	1	0.071428571
map01070	Biosynthesis of plant hormones	0	0	3	0.305194805
2. Genetic Information Processing		0	0	3	0.344877345
2.2 Translation		0	0	3	0.344877345
map00970	Aminoacyl-tRNA biosynthesis	0	0	3	0.344877345
3. Environmental Information Processing		2	0.208333333	9	2.662337662
3.1 Membrane transport		0	0	3	0.924242424
map02010	ABC transporters	0	0	3	0.924242424
3.2 Signal transduction		1	0.083333333	5	1.404761905
map02020	Two-component system	0	0	1	0.071428571
map04071	Sphingolipid signaling pathway	0	0	1	0.333333333
map04024	cAMP signaling pathway	0	0	1	0.333333333
map04022	cGMP-PKG signaling pathway	0	0	2	0.666666667
map04152	AMPK signaling pathway	1	0.083333333	0	0
3.3 Signaling molecules and interaction		1	0.125	1	0.333333333
map04080	Neuroactive ligand-receptor interaction	1	0.125	1	0.333333333
4. Cellular Processes		0	0	1	0.333333333
4.3 Cellular community - eukaryotes		0	0	1	0.333333333
map04540	Gap junction	0	0	1	0.333333333
5. Organismal Systems		3	0.791666667	31	8.173881674
5.1 Immune system		0	0	1	0.333333333
map04611	Platelet activation	0	0	1	0.333333333
5.2 Endocrine system		0	0	9	2.293650794
map04922	Glucagon signaling pathway	0	0	1	0.071428571
map04923	Regulation of lipolysis in adipocytes	0	0	2	0.666666667
map04917	Prolactin signaling pathway	0	0	1	0.111111111
map04921	Oxytocin signaling pathway	0	0	1	0.333333333
map04916	Melanogenesis	0	0	1	0.111111111

Table 4. KEGG PATHWAY compound classification. (page 5)
The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG PATHWAY hierarchy were shown. Due to the large size of the table, there is a duplication with the previous page.

Culture Condition		Syneco		Conv	
Category		#Formulae	Uncertainty Score	#Formulae	Uncertainty Score
All Categories		152	39.32423687	226	56.47841953
5. Organismal Systems		3	0.791666667	31	8.173881674
5.1 Immune system		0	0	1	0.333333333
map04611	Platelet activation	0	0	1	0.333333333
5.2 Endocrine system		0	0	9	2.293650794
map04922	Glucagon signaling pathway	0	0	1	0.071428571
map04923	Regulation of lipolysis in adipocytes	0	0	2	0.666666667
map04917	Prolactin signaling pathway	0	0	1	0.111111111
map04921	Oxytocin signaling pathway	0	0	1	0.333333333
map04916	Melanogenesis	0	0	1	0.111111111
map04924	Renin secretion	0	0	2	0.666666667
map04925	Aldosterone synthesis and secretion	0	0	1	0.333333333
5.3 Circulatory system		0	0	2	0.666666667
map04270	Vascular smooth muscle contraction	0	0	2	0.666666667
5.4 Digestive system		3	0.791666667	8	2.245310245
map04970	Salivary secretion	0	0	1	0.333333333
map04976	Bile secretion	2	0.666666667	1	0.333333333
map04974	Protein digestion and absorption	1	0.125	3	0.344877345
map04977	Vitamin digestion and absorption	0	0	1	1
map04978	Mineral absorption	0	0	2	0.233766234
5.6 Nervous system		0	0	4	0.753968254
map04728	Dopaminergic synapse	0	0	1	0.111111111
map04726	Serotonergic synapse	0	0	2	0.30952381
map04730	Long-term depression	0	0	1	0.333333333
5.7 Sensory system		0	0	4	1.071428571
map04744	Phototransduction	0	0	1	0.333333333
map04744	Phototransduction - fly	0	0	1	0.333333333
map04740	Olfactory transduction	0	0	1	0.333333333
map04742	Taste transduction	0	0	1	0.071428571
5.8 Development and regeneration		0	0	1	0.142857143
map04361	Axon regeneration	0	0	1	0.142857143
5.10 Environmental adaptation		0	0	2	0.666666667
map04713	Circadian entrainment	0	0	1	0.333333333
map04714	Thermogenesis	0	0	1	0.333333333
6. Human Diseases		0	0	14	2.536940837
6.1 Cancer: overview		0	0	5	0.616305916
map05204	Chemical carcinogenesis	0	0	1	0.2
map05230	Central carbon metabolism in cancer	0	0	4	0.416305916

Table 4. KEGG PATHWAY compound classification. (page 6)
The numbers of different chemical formulae (# Formulae) associated with the Syneco- and Conv-distinctive loadings for each category of chemical ontology in KEGG PATHWAY hierarchy were shown. Due to the large size of the table, there is a duplication with the previous page.

Culture Condition		Syneco		Conv	
Category		#Formulae	Uncertainty Score	#Formulae	Uncertainty Score
All Categories		152	39.32423687	226	56.47841953
6. Human Diseases		0	0	14	2.536940837
6.1 Cancer: overview		0	0	5	0.616305916
map05204	Chemical carcinogenesis	0	0	1	0.2
map05230	Central carbon metabolism in cancer	0	0	4	0.416305916
6.4 Neurodegenerative disease		0	0	2	0.444444444
map05012	Parkinson disease	0	0	2	0.444444444
6.5 Substance dependence		0	0	5	1
map05030	Cocaine addiction	0	0	1	0.111111111
map05031	Amphetamine addiction	0	0	1	0.111111111
map05032	Morphine addiction	0	0	1	0.333333333
map05034	Alcoholism	0	0	2	0.444444444
6.10 Infectious disease: parasitic		0	0	2	0.476190476
map05143	African trypanosomiasis	0	0	2	0.476190476

Table 5. Results of KEGG PATHWAY category-wise tests with $p < 0.05$.

The difference of intensity in each KEGG PATHWAY category (column "Category in KEGG PATHWAY") is tested with two-sided Welch's *t*-test and Brunner-Munzel test ("Test"). The signs of *p*-values (in "*p*-Value") represent the magnitude relationship of mean intensity between Syneco and Conv ("Magnitude Relationship"); negative sign means Conv was greater than Syneco, and positive sign means Syneco was greater than Conv. The column "Scale" indicates whether the intensity was used in linear or logarithmic scale for the tests. The intensity value was used both as chemical formula-wise aggregated mean and as separated intensity peaks (indicated as Formula and NA, respectively, in the column "Averaging"). The column "# Formulae" represents the number of chemical formulae estimated in each category.

Category in KEGG PATHWAY		#Formulae	Magnitude relationship	Scale	Test	Averaging	p-Value
1. Metabolism		199	Syneco<Conv	Logarithmic	Brunner-Munzel	Formula	-0.047539053
1.4 Nucleotide metabolism		6	Syneco<Conv	Linear	Brunner-Munzel	Formula	-0.047815399
1.5 Amino acid metabolism		56	Syneco<Conv	Linear	Brunner-Munzel	Formula	-0.017333975
						NA	-0.017517541
				Logarithmic	Welch	NA	-0.018298902
					Brunner-Munzel	Formula	-0.01009549
						NA	-0.017517541
	map00300 Lysine biosynthesis	7	Syneco<Conv	Linear	Brunner-Munzel	Formula	-0.021276803
	map00310 Lysine degradation	7	Syneco<Conv	Linear	Welch	NA	-0.007608044
					Brunner-Munzel	Formula	-0.024977507
						NA	-0.014387325
				Logarithmic	Welch	Formula	-0.016582594
						NA	-0.01523004
					Brunner-Munzel	Formula	-0.00014366
						NA	-0.014387325
	map00330 Arginine and proline metabolism	8	Syneco<Conv	Linear	Brunner-Munzel	Formula	-0.035046884
						NA	-0.02678438
				Logarithmic	Welch	NA	-0.026253273
					Brunner-Munzel	NA	-0.02678438
1.6 Metabolism of other amino acids		17	Syneco<Conv	Linear	Brunner-Munzel	Formula	-0.048187025
1.8 Metabolism of cofactors and vitamins	map00830 Retinol metabolism	2	Syneco<Conv	Linear	Welch	Formula	-0.004966483
				Logarithmic	Welch	Formula	-0.000540986
1.0 Global and overview maps	map01100 Metabolic pathways	127	Syneco<Conv	Logarithmic	Brunner-Munzel	Formula	-0.043560396

Table 6. Results of KEGG BRITE category-wise tests with $p < 0.05$.
The difference of intensity in each KEGG BRITE category (column "Category in KEGG BRITE") is tested with two-sided Welch's *t*-test and Brunner-Munzel test ("Test"). Other notations follow those of Table 4.

Category in KEGG BRITE						#Formulae	Magnitude relationship	Scale	Test	Averaging	p-Value
Compounds and Reactions	Compounds (C numbers)	Phytochemical compounds [BR:br08003]	Terpenoids	Diterpenoids (C20)	Abietanes	2	Syneco<Conv	Linear	Welch	Formula	-0.004966483
								Logarithmic	Welch	Formula	-0.000540986
				Sesquiterpenoids (C15)	Guaianolide	3	Syneco<Conv	Logarithmic	Welch	Formula	-0.042426416
			Phenylpropanoids	Monolignols	Sinapate derivatives	2	Syneco>Conv	Linear	Welch	Formula	0.02822976
							Syneco<Conv	Logarithmic	Welch	Formula	-0.032450817
		Glycosides [BR:br08021]	N-glycosides			3	Syneco<Conv	Linear	Welch	Formula	-0.042604306
		Lipids [BR:br08002]	PR Prenol lipids	PR01 Isoprenoids	PR0109 Retinoids	2	Syneco<Conv	Linear	Welch	Formula	-0.004966483
								Logarithmic	Welch	Formula	-0.000540986
Drugs	Drug information (D numbers)	New drug approvals in Japan [br08318]				4	Syneco<Conv	Linear	Welch	NA	-0.039762524
									Brunner-Munzel	Formula	-0.002578598
										NA	-0.019485081
								Logarithmic	Welch	Formula	-0.014647994
										NA	-0.026673591
									Brunner-Munzel	NA	-0.019485081
		Drugs with new active ingredients				4	Syneco<Conv	Linear	Welch	NA	-0.039762524
									Brunner-Munzel	Formula	-0.002578598
										NA	-0.019485081
								Logarithmic	Welch	Formula	-0.014647994
										NA	-0.026673591
									Brunner-Munzel	NA	-0.019485081
	Drug classifications (D numbers)	Anatomical Therapeutic Chemical (ATC) classification [BR:br08303]	M MUSCULO-SKELETAL SYSTEM			2	Syneco>Conv	Linear	Welch	NA	0.049488163
								Logarithmic	Welch	Formula	0.022529231
				M01 ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS		2	Syneco>Conv	Linear	Welch	NA	0.049488163
								Logarithmic	Welch	Formula	0.022529231
					M01A ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS	2	Syneco>Conv	Linear	Welch	NA	0.049488163
								Logarithmic	Welch	Formula	0.022529231

Same as the LS-PCA with 2014-2019 samples, I identified Syneco-/Conv- distinctive loadings in PCA groups 2014-2017, and 2018-2019. Among the three PCA groups 2014–2017, 2018–2019, and 2014–2019, I identified common chemical formulae that were sufficient for the separation of Syneco and Conv samples with LS-PCA (see Appendix 13, 14, 15, 16, 17, and 18). In all the three PCA, "C13H20O2", "C14H21NO8", "C27H30O16" and "C11H16O2" were the common formulae found to be Syneco-distinctive loadings. Through the metabolome categorization with KEGG databases, these were considered to be heptyloxyphenol, glucosylpyridoxine, rutin, and methylcatechol. Moreover, "C44H34O22", "C9H11NO3", "C5H11N3O2", "C14H16O10", "C21H20O10", "C16H18O8", "C9H11NO2" and "C14H20O3" were detected as common formulae as Conv-distinctive loadings. These were considered to be theasinensin A, l-tyrosine, guanidinobutyric acid, theogallin, isovitexin, coumaroylquinic acid, l-phenylalanine and heptylparaben.

The common 4 compounds of Syneco tended to have larger variance values in Syneco than Conv samples (F-test, $p = 0.0795$), while the common 8 compounds of Conv had significantly larger variance values in Conv than Syneco samples (F-test, $p = 2.576\times10^{-13}$). In addition, epigallocatechin gallate, aromadendrin, pesticide compounds (framprop, aldicarb or butocarboxim), and amino acids were detected as Conv-distinctive compounds (although not detected from all the three PCA groups).

To check for differences between Syneco and Conv samples within each functional category, the two-sided Welch's *t*-test and Brunner-Munzel test were performed in each category of KEGG BRITE and KEGG PATHWAY (KEGG category-wise tests), with the use of total intensity and total logarithmic intensity of Syneco2014–2019 and Conv2015–2019 samples. The mean values of the intensity of the same chemical formula in same category, and each value of the actual measured intensity in same category were used for each test (column "Averaging" in Table 5 and 6). Brunner-Munzel test and t-test were performed for the intensity as it is and the log-transformed case, respectively (column "Test" and "Scale" in Table 5 and 6). Thus, for each category, a total of six test patterns were performed for the combination of the three cases. This is to check for differences in Syneco and Conv when multiple components were combined in the same category, not a single component-by-component test.

As a result, the categories of "1. Metabolism," "1.4 Nucleotide metabolism," "1.5 Amino acid metabolism," "1.6 Metabolism of other amino acids," "Abietanes," "Drugs with new active ingredients," "Guaianolide," "M MUSCULO-SKELETAL SYSTEM," "M01 ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS," "M01A ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS,"

850 “map00300 Lysine biosynthesis,” “map00310 Lysine degradation,” “map00330 Argi-
851 nine and proline metabolism,” “map00830 Retinol metabolism,” “map01100 Metabolic
852 pathways,” “N-glycosides,” “New drug approvals in Japan [br08318],” “PR0109 Retin-
853 oids,” and “Sinapate derivatives,” were significantly different($p < 0.05$) in at least one of
854 the tests (Table 5 and 6). Notably, the results showed that Conv samples expressed sig-
855 nificantly greater total intensity in the categories of amino acids- and nucleotide- re-
856 lated primary metabolites.

2-3-3 Discussion

In the pre-analysis with a Welch's *t*-test and Brunner–Munzel test for each compound, only one and four over 342 detected compound peaks had significant differences between Syneco and Conv samples, respectively, concerning the 5% threshold on the *p*-value. Therefore, the simple comparison of compound-wise intensity does not support any statistically significant differences, since about 17 peaks (5% of 342) could stochastically take *p*-values under the 5% threshold in random data. Considering also biological fluctuations and technical errors, the results of compound-wise statistical tests did not seem relevant enough to require further investigation.

However, the variation between different years for each compound measured with F-test was significantly different between the two categories (for 58 compounds, about 17% of 342), suggesting that the *in cultura* samples had larger yearly variance. This result supports the relative consistency of metabolic profile in ecological optimum compared to the larger deviation in monoculture data, as pointed out in a previous study [Funabashi 2015]. It means that the human intervention under the *in cultura* culture conditions introduces larger fluctuations in the metabolic state of the crop than that under the *in natura* conditions. The observed facts may be relevant to the general relationship between biodiversity and ecological resilience: the *in cultura* culture condition with low biodiversity may behave less stable in the consistency of its metabolic profile because of insufficient ecological interactions that are abundant in the *in natura* culture condition.

Previous studies have predicted that there are statistically invariant features of metabolic profiles that distinguish between *in cultura* and *in natura* culture conditions using a simulation based on the available database [Funabashi 2015]. The principal component analysis in this study, reflecting the LC-MS measurements over five years of repeated production, also supports this notion. The distinctive metabolic profiles were found with respect to the PC3 loading section, which was classified with the ontologies in KEGG BRITE and PATHWAY databases.

As for the KEGG PATHWAY classification of the top 130 PC3 loadings (Table 4), 9 compounds of *in natura* samples and five compounds of *in cultura* samples were categorized into "map01120 Microbial metabolism in diverse environments" (see Appendix 19 and 20). This category is related to the metabolism of microorganisms, which implies that the *in natura* samples were raised in more complex microbiological interactions than *in cultura* samples. In this study, the microbiological interaction of tea plants should mainly come from soil microbiota. Indeed, it is reported that Synecoculture largely promotes soil microbial diversity and activities [Funabashi 2017c]. These results

suggest that the *in natura* samples tend to contain more diverse compounds related to the interaction with soil microorganisms.

Plants are known to synthesize repellent and attractant substances called allelochemicals in competitive and cooperative interactions with other plants and insects [Rice 1974]. Allelochemicals such as alkaloids, phenylpropanoids, steroids, and flavonoids, may be harmful to the human body in excessive amounts, but are known to have anti-inflammatory, anti-cancer, and antioxidant effects with appropriate dose [Middleton et al. 2000]. My results suggest that the *in natura* culture condition associated with rich interactions between species could support a more diverse production of such health-protective compounds.

In terms of the amount of phytochemicals, the total intensity of flavonoids was larger in Conv than in Syneco in the total comparison of 2014–2019 samples. This result was different from the previous study that reported larger flavonoids expression in Syneco 2014–2015 samples [Funabashi and Ohta 2020]. Actually, the total amount of flavonoids was superior in Syneco 2014–2016 to Conv 2015–2016 samples (Appendix 5), but when compared across the entire 2014-2019 sample, this result was reversed. In other words, the amount of flavonoids served as a parameter to distinguish between the two culture conditions until 2016, but not for samples from 2017 onward. One possible reason for this is that flavonoids are affected not only by ecological interactions, but also by physical environmental stresses, and may not be maintained in the same state from year to year as differences in metabolic state caused by different culture conditions.

Among the three PCA groups (2014–2017, 2018–2019, and 2014–2019), compounds such as glucosylpyridoxine, rutin, heptyloxyphenol, and methylcatechol were estimated as common distinctive compounds in Syneco samples. Glucosylpyridoxine, a glycoside of vitamin B6, was detected as an exclusively characteristic compound of *in natura* samples. Furthermore, rutin is known as an allelopathic chemical that improves plant disease resistances and inhibits the growth of other competing plants [Golisz et al. 2007][Yang et al. 2016]. These *in natura* distinctive compounds can be interpreted as the results of the enhanced ecological interactions without fertilizer and agrochemicals, compared to conventional farming.

Vitamin B6 is known to be synthesized by soil microorganisms of the genus *Aspergillus*. It is required for controlling the immune response, and its deficiency is known to cause immune system disorders and a decrease in antibody production [Gross and Newberne 1980][Rail and Meydani 1993]. In recent years, the diversity of human gut microbiota in city environments has been reduced due to the abuse of

antibiotics and pesticides [Bello et al. 2018][Defois et al. 2018][Clemente et al. 2015], and non-infectious immune-related diseases have become a serious issue in many countries around the world. Also, the risk of vitamin B2 and B6 deficiency has been reported in vegan populations [Vudhivai, et al. 1991]. Vitamin B6 and B12 have been reported to be effective in treating Alzheimer's disease [Douaud et al. 2013][Bredesen et al. 2018][Shetty and Youngberg 2018]. Having increased amount of vitamin B6 in a daily consumed beverage, such as tea, may be beneficial for the human health. My results which suggested increased vitamin B6 in the samples cultivated under the *in natura* culture, along with other components, indicate that the connection to human health may be worth further examination in relation to Synecoculture.

On the other hand, theasinensin A, l-tyrosine, guanidinobutyric acid, theogallin, isovitexin, coumaroylquinic acid, l-phenylalanine and heptylparaben were detected as the characteristic compounds common to conventional samples. Amino acids were detected as a strong characteristic of *in cultura* samples. This is in line with the previous study that also reported amino acids were one of the characteristics of the conventionally cultured tea product [Funabashi and Ohta 2020]. The results of the KEGG category-wise tests in Table 5 also support this notion.

The overall results suggest that the distinction between *in natura* and *cultura* conditions only becomes possible at the distribution level of metabolome, beyond single-component comparison. Although this study has the limitation of being limited to comparisons based on field samples from the same region, such an increase in secondary metabolites in tea plants may be an example of a general interaction between the crop and field biodiversity, especially the soil microbiota. I have identified examples where tea plants grown under natural conditions can produce more diverse and abundant allelochemicals than under cultivated conditions. It is known that in the formation of ecological niches, some species are specifically dependent on symbiosis with other species and do not tolerate single isolated culture (e.g. [Begum et al. 2019]). This suggests the existence of a "*in natura* effect" that maintains the coexistence of various species in the natural environment and the associated specific metabolite expression patterns that occur only in the complex interactions of self-organized plant-animal communities.

In general, the health benefits of plant products cannot be adequately assessed by a single component, but need to be considered in the context of a whole diet consisting of many compounds that act synergistically on human health [Liu 2003][Nishi et al. 2017]. This study indicates that aspects of culture conditions, which are directly related to environmental impacts, may act indirectly via multiple components of food on

human health. It is necessary to further examine the quality and culture conditions of the products in a comprehensive manner in assessing the sustainability of food systems to solve the trilemma of health, diet, and environment by expanding the perspective to the context called planetary health, where both human and ecological health must be considered.

2-4 Sensory Evaluation of Bancha (green course tea)

2-4-1 Materials and Methods

Sensory evaluations were performed using Synecoculture and conventional Bancha, the same as those presented in Section 2-3-1, except for 2016 samples. Three grams of tea leaves were extracted in boiled water and served for sensory evaluation. The tea was extracted to the same concentration as Section 2-3, 3.0 g in 1 L, and 1 L of the tea was served to the panelists in equal amounts for each trial.

Teas were served to 5-9 panelists (not trained) on each test in all combinations (in total 16 pairs) by randomly selecting a pair of samples from Syneco and Conv teas (2015, 2017-2019). In total, 12 panelists (11 men and 1 woman with a mean age of 37.33 ± 12.31 years) participated in the trial if they were available in the trial day. There was no specific intention to select panelists, but rather the result of gathering a group of people who were available to participate. Trials were conducted between May 7 and November 12, 2021, once a day for a total of 16 trials.

The panelists, in a single-blind condition, rated which of the two teas was superior in each taste item (Umami, Sweetness, Bitterness, Astringency, Fragrance, Complexity, Mild, Well-balanced, Going down the throat, Clearness of taste, depth of color, Prefence, and Overall). All panelists were native Japanese speakers, and the items described were: "旨味・甘味・苦味・渋味・香り・深み・まろやかさ・なじみ感・のどごし・クリアさ・色の濃さ・好み・総合評価" in Japanese, respectively. Items that could not be rated as superior or inferior were rated as the same. Then a majority vote was taken for all the panelists on each test.

993

Table 7: Results of sensory evaluation.

Each white row represents the results for each sensory evaluation. Panelists made single blind judgments on which tea was superior for each taste item, and the most frequent rating among the panelists was finally adopted as the result. "S" or "C" indicates the superiority of the Syneco or Conv samples to the other, and "-" represents the case of no difference, respectively. "Score" is the sum of the results of the 16 tests over 16 days where "S" is counted as 1, "C" is -1, and "-" is 0. A positive value (>0) indicates that the Syneco samples were superior to the Conv samples in total, and a negative value (<0) indicates that the Conv samples were superior to the Syneco samples in total. The bottom two blue rows show the probability that this result would occur if the "S", "C", and "-" ratings were assumed to be random (i.e. p-value of the null hypothesis): The top blue row shows the probability of being equal or lower than the evaluated score, and the bottom row shows the probability of being greater than the evaluated score.

	Sample		Taste Items												
	Syneco	Conv	#Panels	Umami	Sweetness	Bitterness	Astringency	Fragrance	Complexity, Full-bodied	mild, mellow, well-rounded taste	well-balanced	going down the throat	clearness of taste	Depth of Color	Preference	Overall
Day1	2018	2019	6	C	-	S	-	-	-	-	C	C	-	C	C	C
Day2	2019	2018	6	-	-	S	-	S	C	S	S	S	-	S	C	C
Day3	2017	2017	9	C	C	C	C	-	-	S	S	-	S	C	-	S
Day4	2015	2015	7	S	S	-	C	-	S	S	S	S	S	-	S	S
Day5	2015	2018	5	C	-	C	-	S	S	C	C	C	S	S	C	C
Day6	2018	2018	7	-	-	C	C	C	S	S	S	S	S	C	S	S
Day7	2019	2019	6	-	S	C	C	C	S	S	S	S	S	-	S	S
Day8	2017	2018	7	C	C	S	C	C	C	C	C	C	-	S	C	C
Day9	2017	2019	5	C	C	S	-	C	S	S	S	S	S	S	-	S
Day10	2018	2017	5	C	C	S	S	C	C	S	S	S	S	C	S	S
Day11	2015	2019	5	S	S	C	C	S	-	S	S	S	C	S	S	S
Day12	2018	2015	6	-	C	S	C	-	-	S	S	S	S	C	S	S
Day13	2019	2015	5	C	C	C	C	-	-	C	S	S	S	C	C	S
Day14	2015	2017	5	S	S	C	S	S	-	-	C	-	C	S	S	C
Day15	2017	2015	5	-	-	-	S	C	C	C	-	S	S	C	S	S
Day16	2019	2017	5	C	C	S	-	S	C	S	S	S	S	-	S	S
#S				3	4	7	3	5	5	10	11	11	11	6	9	11
#C				8	7	7	8	6	5	4	4	3	2	7	5	5
#-				5	5	2	5	5	6	2	1	2	3	3	2	0
Which is larger				Conv	-	Syneco	Conv	Conv	Syneco	Syneco	Syneco	-	Syneco	Conv	Conv	-
Score				-5	-3	0	-5	-1	0	6	7	8	9	-1	4	6
The probability that the score will be equal to or less than that value				0.0628932	0.1791632	0.5	0.0628932	0.3797314	0.5	0.9669037	0.9839556	0.9928471	0.9970715	0.3797314	0.8896643	0.9669037
The probability that the score will be greater than that value				0.9371068	0.8208368	0.5	0.9371068	0.6202686	0.5	0.0330963	0.0160444	0.0071529	0.0029285	0.6202686	0.1103357	0.0330963

2-4-2 Results

The results were shown in Table 7. The Conv samples tended to have greater evaluation than the Syneco samples in the items of Umami and Astringency.

The Syneco samples tended to have better evaluation than the Conv samples in the items of Mild/Mellow/Well-balanced, Going down the throat, Clearness of taste, and Overall. There was no significant difference between the Syneco and Conv samples in the items of Sweetness, Bitterness, Fragrance, Complexity/Full-bodied, Depth of color, and Preference.

2-4-3 Discussion

In sensory evaluation, the Conv samples were superior to the Syneco samples in Umami, which was consistent with the previous metabolomic analysis (Section 2-3) that amino acids known as Umami compounds were classified as Conv distinctive compounds. The Syneco samples were superior to the Conv samples especially in Clearness of taste. Clearness of taste can be considered as a comprehensive characteristic beyond specific tastes such as umami, sweetness, and saltiness. This is because umami, sweetness, and saltiness are classified as the five basic tastes to which specific compounds correspond, while Clearness of taste is considered to be perceived for the taste as a whole, not for a specific taste. This was consistent with the previous finding that the *in natura* and *in cultura* culture conditions could only be separated by the overall expression pattern of multiple compounds in metabolome analysis beyond single characteristic compound. In sensory evaluation, as well as in metabolomic analysis, this means that the two culture conditions were more likely to be distinguished in complex combinations of multiple components. These results suggest that well-designed sensory evaluation can qualitatively distinguish the overall state of tea metabolome in terms of *in natura* and *in cultura* culture conditions.

For future application, by refining the sensory evaluation method and combining with metabolomic data using methods such as PLS (partial least squares regression), it may become possible to infer objective metabolites from subjective human evaluations in a simplified manner.

2-5 Effect of Synecoculture products on human well-being

2-5-1 Materials and Methods

From 17th January to 1st April, 2016, 33 Japanese adult female subjects were randomly and equally divided into two groups (Conventional Intervention Group: CIG and Synecoculture Intervention Group: SIG) and asked to spend 3 weeks in each timing as follows. In order to have them live as similar life patterns as possible in each phase, housewives were prioritized as subjects. Only those who were fully informed about the experiment and agreed to its purpose participated. Subjects could withdraw from the experiment at any time.

1. Control phase (C1, C2)
   Subjects will not drink any type of Bancha tea.
2. Intervention phase (IS)
   Subjects will drink 3.0 g of Syneco 2015 infused in 1 l of boiling water per day.
3. Intervention Phase (IC)
   Subjects will drink 3.0 g of Conv 2015 infused in 1 l of boiling water per day.

The temporal order of the control and intervention periods was combined as follows:
Conventional Intervention Group (CIG): 1-week control period C1, 1-week intervention period IC, and 1-week control period C2
Synecoculture Intervention Group (SIG): 1 week control period C1, 1 week intervention period IS, and 1 week control period C2.

The handling of tea samples was double-blinded. These phases were conducted based on the ordinary life pattern of the subjects, which was expected to be consistently reproducible on a weekly scale. Unusual traveling and diet during the experiment were avoided. Regular exercise activities were allowed and practiced in both phases.

The triaxial accelerometer (OMRON Active Style Pro HJA-350IT37) was used to measure the subject's physical activity. It was fixed to the subject's waist, and cumulative triaxial acceleration data were acquired at 1-minute intervals. To avoid subjective bias, no information was shown on the display during the experiment.

Household and locomotive activities were differentiated according to the gravity-deflected physical activity classification algorithm [Oshima et al. 2010]. Household refers to activities of daily living activities such as sitting, doing housework, eating, etc.,

while locomotive activities refer to the state of walking. Physical activity intensity was expressed in metabolic equivalents (MET) and validated with high accuracy for household and locomotive activities [Ohkawara et al. 2011].

Data were extracted and analyzed with BI-LINK PROFESSIONAL EDITION Ver.1.0 [OMRON 2008] using subjects' parameters such as age, body height and weight, which derived estimation of calorie consumption with respect to individual base metabolism [Ganpule et al. 2007]. Household and locomotive calories were distinguished, which together constituted total activity. In the case of METs less than one during 1hr, these were corrected to the mean basal metabolic rate 0.9 MET. I considered the amount of activity to be 0.9 MET per hour when the device was worn and little movement was measured (according to population analysis data, the value is between 0.8-1.3 MET.

Only data from days with at least 600 minutes of wearing time were considered valid measurements. A Box-Cox transformation was applied to the measured values and two-sided paired t-tests were performed. I analyzed the difference between the intervention and control phases with renormalized daily mean calorie consumption, with respect to the mean values of the control C1 phase for each subject.

I also investigated the amount of caffeine ($C_8H_{10}N_4O_2$) and total flavonoids, which have been reported to affect a person's energy expenditure [Stohs and Badmaev 2016], using data obtained in Section 2-3 for Conv 2015 and Syneco 2015.

2-5-2 Results

Valid data were obtained from 27 Japanese female adult subjects between 17th January – 1st April 2016. The CIG and SIG comprise 13 and 14 subjects, respectively. The subjects were distributed on age: 45.778±6.86, height: 157.91±3.92 cm, and weight: 51.159±6.82 kg. Estimated total energy expenditure did not differ significantly in both CIG and SIG during C1 and C2($p=0.438$ and 0.581, respectively, with two-sided paired t-test on individual means).

Table 8 shows the results of the estimated household, locomotive, and those total energy expenditure of CIG and SIG. Significant increase ($p<0.05$) of locomotive activity was observed for SIG ($p=0.0125$, one-sided paired t-test on individual means), while that of CIG did not have a significant trend with $p=0.1890$. The amount of increase of energy expenditure of locomotive activity in SIG was 14.5% when normalized by the mean value of C1 per individual (see Appendix 21). In contrast, household energy expenditure decreased in SIG ($p=0.0464$) and tended to increase in CIG ($p=0.0718$) compared to the control phase, resulting in an insignificant change in total activities.

Table 8. Results of human physical activity measurement in response to green course tea (Bancha) intake.

Sample name and experiment group, subject number and p-value of one-sided paired t-test of locomotive, household, and total (household and locomotive) calorie consumption between intervention phase (I) and control phase (C) are listed. Positive and negative signs of p-values represent the relation of mean energy expenditure between experimental phases, I>C and I<C, respectively. Daily data are available in Appendix 21.

Sample	Syneco2015	Conv2015
Group	SIG	CIG
#subjects	14	13
p-value(locomotive)	0.0125	-0.189
p-value(household)	-0.0464	0.0718
p-value(total)	-0.4609	0.1974

Caffeine intensity was lower in Syneco 2015 (intensity = 1596131) than in Conv 2015 (intensity = 1683899) and total flavonoids intensity was higher in Syneco 2015 (intensity = 3056698) than in Conv 2015 (intensity = 3003226) (see Appendix 4 and 5).

2-5-3 Discussion

There was a significant increase in exercise energy expenditure in SIG compared to the control period, associated with a decrease in household activity. These changes can be summarized as a shift in the composition of activities from housework to locomotive activity or higher exercise such as brisk walking [Oshima et al. 2010]. This trend was reversed in the *in cultura* sample of Conv 2015, but not significantly.

Although this study cannot verify any causal relationship between activity changes and certain components in the tea samples, following speculation of causality based on the previous studies and the data obtained from my results can be made.

Caffeine has been widely studied as a compound that reproducibly affects human physical activity by increasing metabolic rate, energy expenditure, and heat production through sympathetic activation. Caffeine was rather more abundant in Conv 2015 than in Syneco 2015. This indicates that the increase in physical activity in Syneco 2015 cannot be explained solely by the amount of caffeine in the sample.

Flavonoids are known to promote energy metabolism and sports performance [Stohs and Badmaev 2016][Huang et al. 2014], and the higher total flavonoid content in Syneco 2015 is one possible explanation for the change in activity. However, it should be noted that in Sections 2-3, total flavonoid content is not mentioned as a characteristic character of the two culture conditions.

A recent rehabilitation experiment [Funabashi 2022], which used a 2016 tea samples, reported that *in natura* coarse green tea significantly improved subjects' rehabilitation performance under a double-blind experiment. This was the result of drinking both teas in conjunction with rehabilitation over a period of four months, and in a comparison by a total population of more than 110 people. Only the group drinking the Syneco 2016 sample improved a measure called FIM (functional independent measure) and PGCMS (Philadelphia Geriatric Center morale scale) in rehabilitation. FIM is a measure of daily activities such as dressing, toileting, and transferring, which a normal person would normally achieve a perfect score on. PGCMS is a measure of human well-being, which is measured by questionnaire. Tea may have various effects on human performance, not only on the amount of activity, which is the focus of this study.

The effects of the *in natura* products on consumers may differ somewhat depending on the culture conditions, and the *in natura* products may be more effective as far as the present results are concerned, but the mechanism of action is still unknown. Further large-scale studies would be necessary.

2-6 Conclusion

In the analysis of fatty acid composition, n-6/n-3 ratio differed between *in natura* and *in cultura* samples in arugula and Bancha. Metabolome analysis revealed characteristics of both culture conditions in Bancha samples produced during 2014-2019.

Although these findings cannot be treated as generalized phenomena due to the limited number of samples and the region of origin, even when various errors are taken into account, the same trend was observed when the samples were divided into two groups based on the criterion of culture condition. From the results of the sensory evaluation, it was inferred that there were consistent taste characteristics in each of the two culture conditions, even though they were produced in different years.

In addition, as far as a simple interpretation of the fatty acid and metabolome results is concerned, it can be inferred that *in natura* products have a better impact on human health in the samples of this study, as shown partly in the activity shift in this

study. These results were in line with the other studies which also showed a positive impact of *in natura* products on human health.

These findings suggest that the metabolic state of the products differs depending on the qualitatively different conditions, i.e., *in natura* and *in cultura*, and that the effects of the products on humans also differ. These results indicate that culture conditions, an aspect of food production that links environmental impact and human health, is a important area of research.

3: The effectiveness of subjective evaluation by humans

3-1 Introduction

Synecoculture produces a high-density mixture of crops and useful plants to achieve a state of augmented biodiversity and ecosystem function, i.e., an augmented ecosystem. Although there have been successful examples in rural areas as described in Chapter 1, there are few examples of such augmented ecosystems intentionally introduced in urban areas, where urbanization is another major factor in global biodiversity loss. It is also not yet clear how augmented ecosystems can contribute to ecosystem services in cities that use relatively small and fragmented ground surfaces that are disconnected from groundwater, such as rooftop gardens and planters. Also, as previously mentioned, there are numerous considerations for the practice of Synecoculture, and the practitioner's ability to understand the ecosystem is also important.

Therefore, in this study, an augmented ecosystem was established in planters and small ground areas on the rooftops of urban buildings, and soil microbiological and chemical analyses, as well as subjective human assessment, were conducted. The main objective is to obtain data on one aspect of soil change in augmented ecosystems and its relationship to the associated human assessment to see if the practices contribute to the acquisition of subjective competence to properly assess and manage augmented ecosystems.

3-2 Materials and Methods

Starting from March 2019, I implemented an augmented ecosystem on the rooftop of six-story building at Roppongi Hills in Tokyo, Japan, by introducing a large number of edible plants into a soil plot (hereafter I call it “Field”) of approximately 40 m^2. Five regular hexagonal planters with a side length of about 70 cm were also placed slightly apart on the same rooftop (Figure 5). In total, at least 110 species were introduced at the time of initial installation, and the augmented state of ecosystem was maintained by continuously introducing edible and useful plants based on the Synecoculture Manual [Funabashi 2016a]. Harvesting of edible plants and mowing of unnecessary biomass were also exercised aiming for positive disturbance on biodiversity. The five planters were designated as A, B, C, D, and E, respectively, and different initial conditions were set as shown in Table 1, with the difference in soil type, tree species, and the selection of other herbaceous species. Based on the manual, each person (X, Y, Z) selected seeds and plants to introduce in each one’s assigned planter, performed the actual maintenance work, and harvested and managed the plants at their discretion during the two-year experiment. There were no restrictions on the

acquisition of relevant knowledge outside the practice, and on the actual work as long as it fits the scope of the manual. There was no incentive to deliberately make good or bad conditions for each planter, i.e., the basic strategy was to try always improving the whole planter ecosystems. In addition to X, Y, and Z, various other people who had learned Synecoculture methods assisted the management in the Field plots.

Figure 5. Example of Synecoculture planter and Field site.
The photo was taken in July, 2020. (a) Planter C after the continuous management of Synecoculture. (b) A view of the placement of the five planters before the installation of edible plants. (c) A view of the placement of the five planters after the installation of edible plants in May 2020. (d) Field plot with edible plants planted directly in the one-meter-deep soil on the roof. More than 20 species of fruit trees were introduced into the Field, and they continued to be introduced

according to the management protocol and availability at local gardening store, without intentional preference concerning the difference between vegetable, herb, and flower.

Table 9. Experimental condition of five planters.

Planter	*Soil type*	*Center tree*[*1]	*Other plants*[*2]	*Manager*[*3]
A	Humus soil[*4]	Laurel	Mainly flowers	Person X
B	Perlite soil[*5]	Blueberry	Mainly vegetables	Person Y
C	Humus soil	Blueberry	Mainly vegetables	Person Y
D	Akadama[*6]	Blueberry	Mainly vegetables	Person Y
E	Humus soil	Mandarin orange	Mainly herbs	Person Z

*1 Center Tree: A tree planted in the center of a planter.

*2 Other Plants: Main introduction strategy of the groups of useful plants to be planted around the tree.

*3 Manager: The person who was in charge of the planter.

*4 Humus Soil: Black soil topped with humus of about 5 cm deep. No fertilizer was used.

*5 Perlite Soil: Light soil made of baked perlite, often used for rooftop gardening [Papadopoulos et al. 2008]. No fertilizer was used.

*6 Akadama: Red ball earth, clay-like soil. No fertilizer was used.

A total of 20 soil samples (three samples from each of the five planters and five samples from the field), were collected in January 2020 and February 2021, and analyzed for soil microbial diversity and vitality value (hereafter SMDVV) [Sakuramoto et al. 2010] and chemical properties (electrical conductivity, pH, effective phosphoric acid, exchangeable lime, exchangeable magnesium, exchangeable potassium, humus, cation exchange capacity (CEC), lime saturation, magnesium saturation, potassium saturation, base saturation, lime magnesium ratio, and magnesium potassium ratio). The SMDVV is a numerical value calculated by comprehensively analyzing how much the 96 predefined compounds can be decomposed by microorganisms in the soil. The 96 compounds were prepared on a single plate, to which soil extracts were dropped, and the color change of the decomposed wells was analyzed using images to measure the number of compounds decomposed and the speed of decomposition. A high value provides relative measure that reflects the degree of microbial diversity and vitality combined [Sakuramoto et al. 2010]. Although a high value of SMDVV does not

necessarily mean that the soil is in good condition, it can be understood that a high value indicates a high degree of microbial diversity and activity, and positive effects such as less incidence of soil diseases have been reported [Sakuramoto et al. 2010]. Based on the assumption that chemical properties, such as the concentration of a single mineral component, are assumed to be difficult to change in a short period of time without artificial fertilizer inputs, while biological properties are relatively easy to reflect the management of aboveground vegetation, I tentatively treated this indicator as a representative indicator of changes in soil richness in this study.

As a method of sampling three points from each planter, each person (X, Y, Z) selected a sampling point where the soil condition seemed to be 1) good according to their experience, or conversely, 2) a point where the soil condition seemed to be bad, based on a comprehensive subjective assessment of plant diversity, growth and estimated rooting rate (these are expressed as 1): +++ and 2): +, respectively, in Table 10). In addition to those good and bad points, the soil was collected from at least three other points randomly and mixed well, which was expressed as ++ in Table 10. Thus, the sampling was done from each planter, based on the three relative levels (+, ++, +++) based on the human subjective evaluation. From the Field, a total of five points were sampled: three points from the center of the ridges (production area in the Field) covered with vegetation, one point from the pathway (non-production area in the Field) with less vegetation and being trampled regularly, and one point from the edge of the Field where no plants were growing. Thus, in the planter, the sampling points in 2020 and 2021 could be different, but in the Field, they were taken from the same location.

I examined the qualitative agreement between this human assessment and SMDVV, a one-dimensional measure of soil microbial diversity and activity. I also analyzed whether chemical properties of five planters qualitatively matched human subjective evaluation on soil quality. For SMDVV, it seems more reasonable to assume that the higher the value, the better the condition of the soil, but for the other chemical properties, I also examined the reverse agreement to consider the possibility that the lower the value, the better the soil. For the sake of clarity, SMDVV was used in form of its deviation value, which means how well the SMDVV was positioned in the population data from 7,000 sites throughout Japan sampled by DGC Technology Inc. [DGC]. A deviation value of 50 means that it is equal to the mean of the population.

I also investigated whether the values of SMDVV correlated with those of other chemical property indices. In addition, PCA was performed using the soil chemical property to visualize the changes over a one-year period with Synecoculture practices.

3-3 Results

All results are shown in Table 10 and Figure 6.

Table 10. Results of soil microbiological and chemical analyses and human subjective evaluation.

The top is the result of the planters and the bottom is the result of the Field. The results of all other chemical analyses are in Appendix 22.

Planter	A			B			C			D			E		
Sample ID	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3	D-1	D-2	D-3	E-1	E-2	E-3
Soil evaluation by human observation	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++
2020 Deviation Value of SMDVV	65.3	55.1	60.6	48.7	54.1	61.0	53.9	63.5	65.5	41.4	43.2	50.6	59.4	64.0	52.0
2021 Deviation Value of SMDVV	66.3	57.2	60.4	61.7	59.9	67.4	62.9	61.4	61.1	55.1	47.9	51.5	70.2	59.0	65.2
2021 CEC (me/100g)	65	67	66	34	47	71	70	71	65	49	49	50	67	70	67
2021 Humus (%)	7.7	8.9	8.3	2.2	2.0	2.5	10	8.2	15	2.1	1.6	2.0	10	8.9	11

	Field				
Sample ID	F-1	F-2	F-3	F-4	F-5
Ridge	Ridge 3	Ridge 5	Ridge 7	NA (Aisle)	NA (Non-vegetation)
2020 Deviation Value of SMDVV	57.3	63.5	50.9	52.0	56.0
2021 Deviation Value of SMDVV	68.4	58.2	69.2	57.0	55.0
CEC(me/100g)	60	59	61	54	47
Humus(%)	7.4	7.0	7.2	8.2	8.6

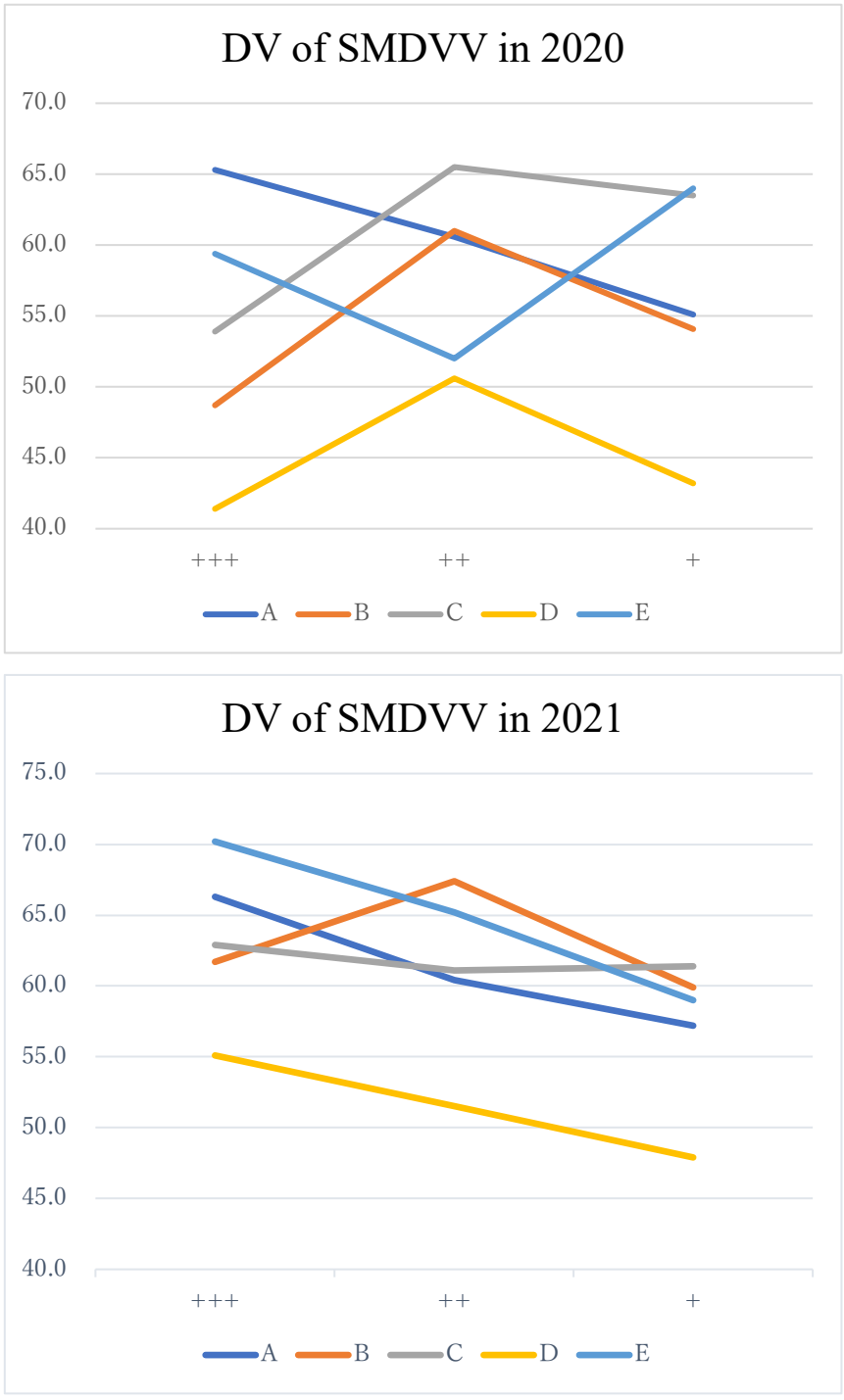


Figure 6. Graphical representation of Table 10.
Deviation Value of SMDVV (vertical axis) is plotted for the planters A-E with the three orders of subjective evaluation (+++, ++, + in horizontal axis). Top: Results of 2020. Bottom: Results of 2021.

From 2020 to 2021, the mean value of the deviation value (DV) of SMDVV significantly increased from 55.9 to 60.75 (p-value= 0.00572, two-sided paired t-test).

The soil evaluation by human observation has improved over the course of 2-year practices: In 2020, only Planter A had the subjective evaluation that qualitatively matched the actual objective measurement; the probability of matching more than one of the five planters was $1-(5/6)^5=0.598$, indicating that this could often happen even if the evaluation was random. On the other hand, in 2021, subjective evaluation of A, D, and E qualitatively matched the objective measurements; the probability of matching three or more of the five based on the random hypothesis was 0.0355. This is a level that can be regarded as a significant match if the statistical significance level is set at 5%.

In addition to SMDVV, I examined the relationship between these data and human subjective ratings in 2021 and found that pH, exchangeable lime, lime saturation, and base saturation were in similar or better agreement with SMDVV. These values were higher the better the soil was rated by human subjective evaluation. In other words, the soil that was rated worse with respect to pH was on the more acidic side. Conversely, the reversed relationship was found only for humas, with the soil rated as better by subjective human evaluation having lower values. All data were shown in Appendix 22.

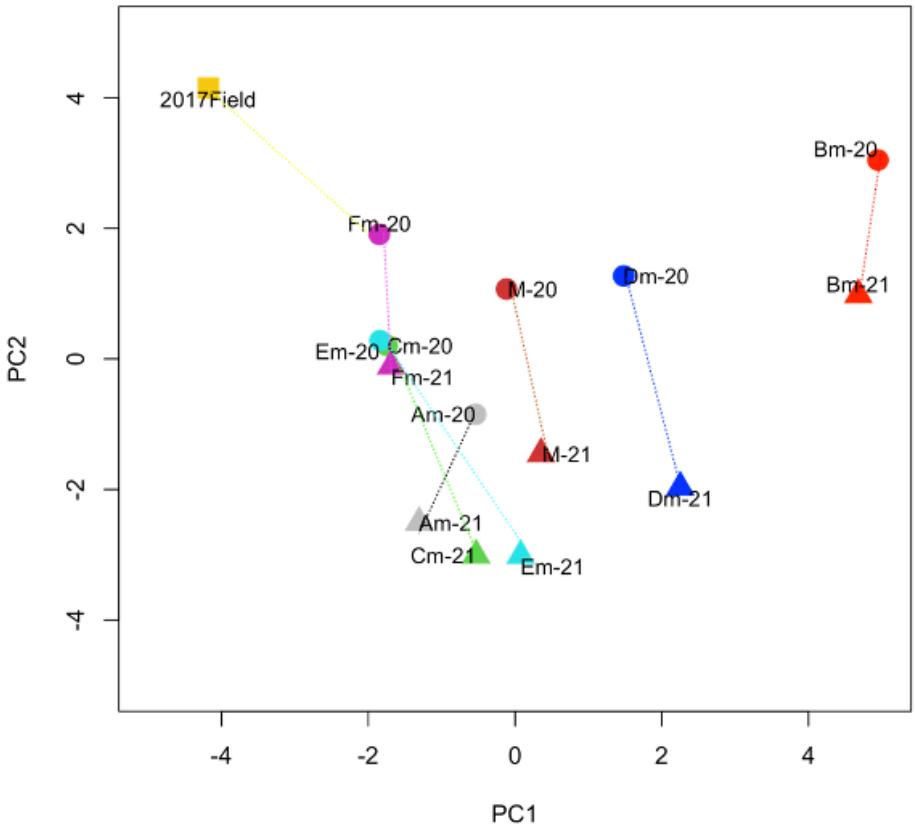


Figure 7. Results of principal component analysis.
PC1-PC2 plane is shown. Am, Bm, Cm, Dm, Em, and Fm represent the average of the planters A-E and Field data, respectively. The numbers represent the year of data acquisition, and the circles correspond to the measurement in January 2020, and the triangles to that of February 2021. M represents the average value of the whole data in each year. 2017Field is the Field data taken in December 2017 before the start of the experiment in March 2019. Temporal change is shown as the dotted lines of the same color.

Principal component analysis was performed with the soil chemical data (Figure 7). There exists global tendency that sample values move from the second to fourth quadrant on PC1-PC2 plane through yearly change, i.e., from negative to positive in PC1 and positive to negative in PC2.

The top three contributions to PC1 were: exchangeable lime (eigenvector: -0.399); base saturation (-0.382); lime saturation (-0.381). The top three contributions to PC2

were: exchangeable potassium (eigenvector: -0.398); electrical conductivity (-0.392); pH (0.383).

In terms of the correlation with soil microbial diversity and activity, only humus (PC1:-0.851, PC2: 0.0390) and CEC (PC1: -0.450, PC2: 0.496) data in 2021 were significantly and positively correlated with SMDVV data in 2020 (Pearson's correlation coefficient r=0.644, p=0.00220 for humus, and r=0.534, p=0.0153 for CEC). The SMDVV in 2021 also showed a similar trend of correlation with humus and CEC but remained at insignificant level.

3-4 Discussion

The SMDVV analysis result suggests that maintaining the diversity of aboveground vegetation based on the Synecoculture method in this experiment has contributed to a significant increase in the diversity and vitality of soil microorganisms from 2020 to 2021. However, this does not mean that only Synecoculture has such effects, considering that monoculture can also improve SMDVV [DGC]. It should be noted that the average SMDVV value in Synecoculture farm was previously reported to reach 72.4 in another open-field experiment in Tokyo [Funabashi 2017c], which is higher than my result, suggesting that there is still a possibility for increasing this value through continued practice.

The human assessment compared with the SMDVV and other chemical properties results suggest that the first-person experience of Synecoculture in an urban environment has improved the accuracy of human evaluation on soil microbial diversity and vitality. In addition to SMDVV, the agreement with indicators such as pH and exchangeable lime also implies a tendency for soils to be relatively more acidic when they are judged to be poor soil, suggesting that changes in aboveground vegetation due to acidification may be affecting human evaluation.

The yearly changes shown in PCA plot (shift downward in PC2 plane) implies that there exists a direction of change in soil chemical property associated with the development of complex vegetation through the practice of Synecoculture without the application of plowing and inputs such as fertilizer and agrochemicals. In this experiment, there were four types of soil, three in the planter and one in the field, and this change was observed in all types of soil; the results were consistent even if the practitioner, soil, and introduced vegetation are different in this experimental environment. Further large-scale studies are needed to determine the reasons for and implications of this change.

I also checked whether CEC and humus of five planters qualitatively matched human subjective evaluation on soil quality, because higher CEC and humus are preferred soil properties for agriculture. Only one (planter C for CEC and D for humus) out of the five planters matched correctly. Combined with the fact that human subjective evaluation matched well with the SMDVV values, the results imply that CEC and humus are relatively more influenced by planter conditions such as soil type, and human observation was not able to accurately predict these type of soil chemical properties in contrast to the soil microbial diversity and activity.

Overall, the constant augmentation of soil microbial diversity and activity was observed even in a city environment where interaction with the surrounding ecosystem is considered to be limited compared to natural environment. The human assessment results suggest that it may be possible to intentionally train human managers in their assessments of soil quality. For example, it may be possible to learn by obtaining more data like photographic records and soil samples, and then ranking them more closely and comparing them to the results (objective soil data). This opens the possibility to count on the development of human ecological literacy through the augmentation of ecosystems to complement the environmental assessment in a pragmatic way.

This experiment had the limitation of having only three practitioners, which was treated as a case study, but the obtained results support the rational of Synecoculture that goes in line with the self-organizing process of ecosystem towards ecological optimum [Funabashi 2016b]. Also the results further clarified that the essential complexity underlying the development of both aboveground vegetation and soil microbial communities cannot be simply reduced to a single chemical property. On the other hand, comprehensive human experience may contribute to establish a capacity to qualitatively judge the desirable direction of ecological augmentation processes. The integrated assessment incorporating both precision measurements of various environmental parameters and empirical human competence in wholistic evaluation could be expected to realize more cost-effective, interactive and flexible ways for ecosystem management. Since this experiment is more of a case study, but it is hoped that this will be an opportunity to explore ways to efficiently evaluate ecosystems by combining human evaluation and objective indicators of soil.

4: Ecosystem Navigation

4-1 Introduction

Measurement in scientific research generally has two essential aspects: 1) the measurement of the part, which assesses the accuracy of the elementary pieces that make up a model, and 2) the measurement of the whole, which assesses the validity of the results of a model applied to explain an observed phenomenon.

When addressing the management of "open complex systems" underlying global sustainability challenges such as climate change, biodiversity loss, and human well-being, feedback between the two different scales of the target system, the part and the whole, becomes particularly important [Tokoro 2017]. Especially in the area of food production, where environmental impacts and their recovery are of concern, the multiple tradeoffs between health, diet, and environmental quality are inherently intertwined, making it difficult to establish a single objective scale for assessing and steering the diverse aspects embodied in dynamic operational processes [Funabashi et al. 2017]. To adequately address these issues, multiple comparative measures need to be established, ranging from one-way causal relationships between single elements to system-level interactions, based primarily on the temporal reconstruction of system boundaries and structures in an open environment [Funabashi 2018]. These are characterized by the fact that the target domain is open, the boundaries of the system cannot be completely defined, its function and structure change from equilibrium over time, its dynamics are not reversible and historically reproducible, it is not completely partitioned into independent subsystems, and it is limited to observations from within the system [Funabashi 2018]. More broadly, it emphasizes the characteristics of being a complex system [Tokoro 2017][Funabashi 2018].

Compared to conventional agriculture, which is an open system but requires minimal interaction with other species through the use of tillage and agrochemicals to fertilize a single crop, Synecoculture is a more complex system because of the relationships between numerous plant species and the other species that interact with them (animals, fungi, etc.), the environment created by the coexistence of various plant species (sunlight, water content), the diversity of human interventions (weed management methods, spatial and temporal arrangement of species to be introduced, etc.). For such methods to be successful, it is important to constantly update appropriate evaluation methods by feeding back the results of partial and total measurements to each other, leading to more efficient management.

The Figure 8 schematically represents sailboat-type navigation system that drifts to its destination under the influence of the outside world, based on a comprehensive

understanding of the ever-changing wind and wave movements over time. Synecoculture does not artificially intervene strongly in environmental conditions, as is the case with tillage, fertilizers, and pesticides in conventional farming methods, but develops the ecosystem through the input of plant resources. This can be represented by this sailboat-type navigation system. Synecoculture does not artificially improve the soil, but requires the appropriate introduction of seeds and seedlings to achieve ecosystem development, including soil, while reading the existing vegetation and environmental conditions at the site.

In this chapter, I examine the feasibility of ecosystem navigation by defining three major ways of operating ecosystems to put this navigation concept into practice and categorizing the accompanying ecosystem responses in order to address Synecoculture as an open complex system management. I also examined the possibility of replacing invasive measurements with non-invasive, low-cost measurements for this purpose.

This corresponds to sensing and managing complex ecosystems with simpler methods, in contrast to what is known as precision agriculture, where state-of-the-art sensing systems are developed to make conventional farming methods more efficient. In order to realize sustainable agriculture, it is necessary to understand and properly manage ecosystems in most fields using methods that do not burden the environment, while using the power of big data and ICT.

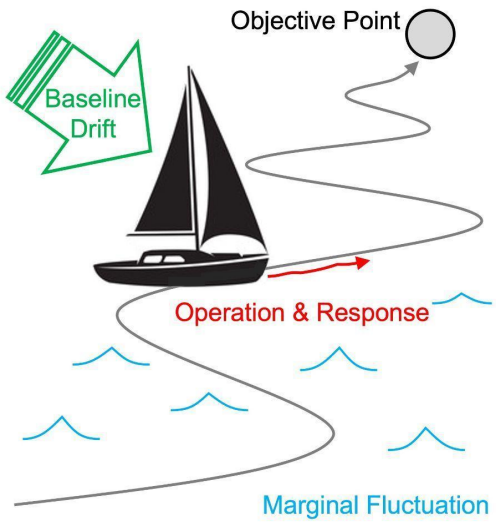


Figure 8. Typology of navigation in open complex systems.
Sailboat-type navigation that cannot avoid large environmental influences but is possible to navigate with fewer resources based on the smart sensing and harnessing of diverse situations in open complex systems.

4-2 Materials and Methods

4-2-1 ABC navigation model

I extract three typical biodiversity operations in the Synecoculture method as the steering wheel of sailboat-type navigation, namely the ABC navigation model (Figure 9): Starting from a given stage of the land ecosystem, the operation A for augmentation represents the introduction of useful plant species to enhance their productivity with various associated ecosystem services; the operation B for baring of the land is to physically remove all aboveground biomass of naturally occurring species, typically known as weeding; and the operation C for conservation leaves the field intact from any human intervention and let the ecological succession proceed as a matter of self-organizing process. The combination of the operations A, B, C has the capacity to navigate the aboveground ecosystem in different directions, which could 1) augment various ecosystem functions (A and C); 2) increase the dominance of useful plant species compared to naturally occurring ones (A and B); 3) create monoculture situation (continuous application of B and limiting A to a single crop); and 4) let the natural succession happens that leads to the climax phase in the long run (C with possibilities of adding partial disturbance by A and B that may accelerate the process).

These ecological dynamics would successfully happen if the A/B/C operations trigger the functional changes in soil ecosystems that comprise interactions between physical, chemical, and microbiological components [Funabashi 2017c][Funabashi and Minami 2021]. In the analogy to sailboat type navigation, the A/B/C operations are the steering wheel, and the responses of subjectively measured plant diversity measures correspond to the actual movement of the rudder, and the effective changes in objective soil measures are the actual movement of the sailboat in response to the steering. In order to assess the effectiveness of the ABC navigation model, I investigated the actual changes in aboveground species composition and associated soil variables through the field experiments.

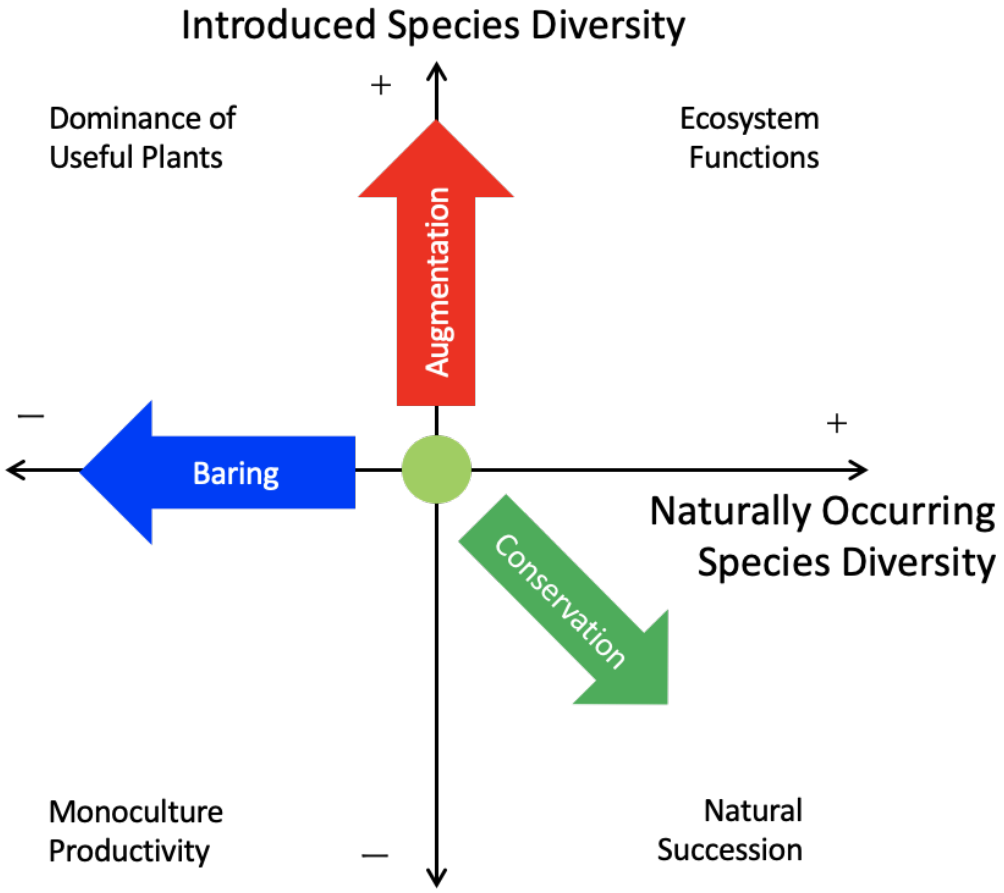


Figure 9. Schematic representation of the three operations A/B/C on plant species diversity.

X-axis stands for the relative increase (+) and decrease (-) of naturally occurring plant species, and Y-axis for the introduction (+) and disappearance (-) of useful plant species, starting from the current status of biodiversity in an ecosystem (light green circle).

A (red arrow): Augmentation of the ecosystem through the introduction of useful plant species.

B (blue arrow): Baring of land by intentional removal of naturally occurring species to protect concurrent introduced species.

C (green arrow): Conservation that refrains the field from any biodiversity operation and leaves it to natural succession. It also corresponds to the control of experiment in contrast to the operations A and B.

The combinations of A/B/C have the potential to navigate the system state to attain the different goal states in the long term, such as the enhanced level of multiple ecosystem functions (top right); increased dominance of useful plants (top

left); maximization of monoculture productivity (bottom left); and the climax stage of natural succession (bottom right).

4-2-2 Field experiments

Synecoculture experiment at Todoroki farm, Tokyo (experiment T)

I established a Synecoculture farming ecosystem in an urban ground at Todoroki, Setagaya-ku, Tokyo on 250 m^2 surfaces through the practice of four years from April 2011 to March 2015, following the Synecoculture manual [Funabashi 2016a] (Figure 10 left). Agriculture had been practiced prior to this experiment, so it was not in a state of natural neglect. In total, more than 35 species of fruit trees were strategically introduced according to the local condition of sunlight and soil moisture, and more than 150 species of vegetables and edible herbs were introduced with seeds and seedlings, without the application of tillage, fertilizer, and agrochemical. I practiced occasional harvesting of vegetables, herbs and fruits as well as weed control by partial mowing. The definition of weed is basically a non-edible species, but sometimes edible species were also mowed depending on the state of the ecosystem.

During the experimental period from March 28th to June 27th, 2015, I selected 36 circular spots of 0.5m diameter in the experimental field and divided them into three groups on the first day (12 spots for each of A/B/C operations), and performed the assigned operation to each spot at monthly intervals (on March 28th, April 26th, May 30th, 2015). Those spatial configurations were planned to maximize the effects of operation based on the farming experience [Funabashi 2017c], that is, the practitioner selected operation A, B, or C for each spot, the operation that best matched the development of that local ecosystem. To the 12 spots chosen for the Augmentation, the seeds of 50 vegetables and herbs that were commercially available were introduced to maximize the coexistence of introduced plants. In the 12 Baring spots, aboveground parts of naturally occurring plants other than introduced ones were repeatedly removed. The 12 Conservation spots have remained untouched since the beginning of experiment , with no intervention. During the experiment, I measured 108 Variables including the Variants (26 variables in Table 11 without the Variants). Variables refers to the main indicators measured, and variants refers to derived indicators based on variables, such as differences in the dates of variables or new indicators created by combining the numerical values of variables.

Synecoculture experiment at Roppongi rooftop garden, Tokyo (experiment R)

Following the same method as the experiment T, I established a Synecoculture farming ecosystem on the rooftop of a six-story building at Roppongi, Minato-ku, Tokyo on a 40 m^2 surface that contains 1m-deep soil (Figure 10 right). Some crops had been produced on the 40 m^2 surface prior to the start of this experiment, but when I started the intervention, there was no vegetation, and the soil was bare. Through 2-year practice from March 2019 to October 2021, more than 187 species of vegetables and herbs were introduced with seeds and seedlings, as well as 24 fruit tree species and were maintained under occasional harvesting and partial mowing.

During the experimental period from October 8th, 2021 to January 7th, 2022, I regularly selected 33 circular spots of 0.5m diameter and randomly applied the operations A/B/C to 11 spots each (on October 8th and 21st, November 4th and 18th, and December 3rd and 15th in 2021). Those special configuration was planned with the Latin square design to randomize the effects of operation and location, such as edge effects of the ecosystem with respect to the variability of spot condition. To the 11 Augmentation spots, the seeds of 35 vegetable and herb species that were commercially available were introduced. In the 11 Baring spots, the aboveground parts of naturally occurring plants were repeatedly removed. The 11 Conservation spots were kept intact from any intervention except automatic watering applied to all 33 spots. During the experiment, I measured 328 variables including the variants (42 variables in Table 11 without the variants), with a partial overlap of 86 variables with T. The variants of the variables were obtained from T and R measurements by calculating the proportions and differences between comparable variables. The details of the measurement methods are explained in Appendix 23.

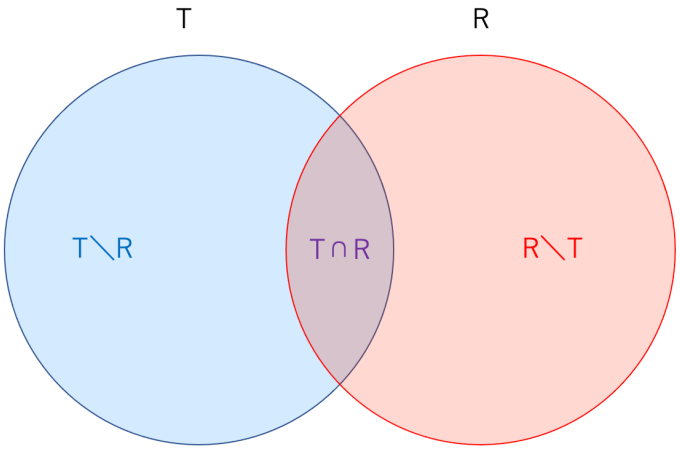
Figure 10. View of Synecoculture farming ecosystems and examples of the A/B/C operation spots in the experiment at Todoroki in spring-summer (left) and Roppongi in fall-winter (right).
Pictures were taken during the experimental period (late April 2015 for Todoroki and mid-November 2021 for Roppongi).

Table 11. List of the variables measured in the experiments T and R.
(Table legend is available in next page)

Variable	Attribution	Invasiveness	Objective/Subjective
Total Carbon	T⟍R	Invasive	Objective
Total Nitrogen	T⟍R	Invasive	Objective
Decomposition Rate (k)	T∩R	Invasive	Objective
Stabilization Factor (S)	T∩R	Invasive	Objective
Permeability(Saturated Hydraulic Conductivity)	T∩R	Invasive	Objective
Soil Microbial Diversity and Vitality Value(SMDVV)	T∩R	Invasive	Objective
Deviation Value of SMDVV	T∩R	Invasive	Objective
Soil Weight just after Sampling	T∩R	Invasive	Objective
Solid Phase Ratio	T∩R	Invasive	Objective
Gas Phase Ratio	T∩R	Invasive	Objective
Liquid Phase Ratio	T∩R	Invasive	Objective
Water Retension Time Scale	T∩R	Invasive	Objective
Humus	T∩R	Invasive	Objective
Soil Weight after Drying	T∩R	Invasive	Objective
Nitric Acid	T∩R	Noninvasive	Objective
Soil Hardness	T∩R	Noninvasive	Objective
Electrical Conductivity (EC)	R⟍T	Invasive	Objective
Ammoniacal Nitrogen	R⟍T	Invasive	Objective
Nitrate Nitrogen	R⟍T	Invasive	Objective
Effective Phosphoric Acid	R⟍T	Invasive	Objective
Exchangeable Lime	R⟍T	Invasive	Objective
Exchangeable Magnesium	R⟍T	Invasive	Objective
Exchangeable Potassium	R⟍T	Invasive	Objective
Cation Exchange Capacity	R⟍T	Invasive	Objective
Base Saturation	R⟍T	Invasive	Objective
pH (H2O)	R⟍T	Invasive	Objective
pH (KCl)	R⟍T	Invasive	Objective
pH	R⟍T	Noninvasive	Objective
K	R⟍T	Noninvasive	Objective
Salt	R⟍T	Noninvasive	Objective
Ca	R⟍T	Noninvasive	Objective
Na	R⟍T	Noninvasive	Objective
Conductivity	R⟍T	Noninvasive	Objective
#Plant Species	T∩R	Noninvasive	Subjective
#Herbaceous Species	T∩R	Noninvasive	Subjective
#Woody Species	T∩R	Noninvasive	Subjective
#Introduced Species	T∩R	Noninvasive	Subjective
#Spontaneous Species	T∩R	Noninvasive	Subjective
#Edible Species	T∩R	Noninvasive	Subjective
#Nonedible Species	T∩R	Noninvasive	Subjective
Lambda Plus (index of variation in taxonomic distinctness)	T∩R	Noninvasive	Subjective
Delta Plus (average taxonomic distinctness for presence / absence data)	T∩R	Noninvasive	Subjective
Shannon Entropy (represents diversity of succession stages)	T∩R	Noninvasive	Subjective
Grading of Ecosystem	R⟍T	Noninvasive	Subjective

Table 11. List of the variables measured in the experiments T and R.

Invasiveness and objective/subjective parameters define the property of measurement methods. Attribution of the variables refers to the set of measured experiments: T∩R means that the variable was commonly measured in both of the experiments T and R, while T⧹R and R⧹T signify that the variable was measured exclusively in either of the experiments T or R, respectively. Complete information with references to the variants and measurement methods excluded in this table is shown in Appendix 23.



T∪R represents all variables of T and R, T∩R the common variables in T and R, and T⧹R and R⧹T are the ones exclusive to T and R, respectively.

4-2-3 Dynamical Assessment and Multivariate Complementary Analysis (DA-MCA)

I applied dynamical assessment (DA) [Funabashi 2017a][Funabashi and Minami 2021] and multivariate complementary analysis (MCA) [Funabashi 2017b] to the field experiment data in order to detect the system-level differences triggered by the A/B/C operations. The analysis took the following steps, in which numerical calculation was performed using the programming language R version 4.1.0 (released 2021-05-18)[CRAN 2022] and the "stats" package version 4.1.0:

Step1. Variable measurements: Perform experiments T and R and obtain datasets using the measurement methods defined in Appendix 23.

The aboveground plant diversity variables are subjective measures observed on-site by humans, and underground soil variables are objective measures obtained through the laboratory analysis of the soil and liquid samples, which follows the definition of subjective and objective measurement in [Funabashi 2017b]. All variables were treated with the Box-Cox transformation to maximize the normality of the distribution before the analysis, i.e., $B(x) := (x^{\lambda} - 1)/\lambda$ for the variable x, which was calculated with the bcPower() function and the optimization of the $\lambda > 0$ parameter using the powerTransform() function in the "car" package version 3.0-12 [Box et al. 1964]. I linearly shifted the raw data to take positive values by adding the minimum value plus 1 before calculating the Box-Cox transformation. I also examined the correlation between variable "Grading of Ecosystem" and other soil indicators at the beginning of Experiment R (row 45 of Appendix 23).

Step 2-1. PCA: To extract generative indices of DA [Funabashi 2017a], analyze the data of T and R using principal component analysis (PCA) with the function prcomp(scale=TRUE) (Figure 12 and Figure 13). Generative indices are those that emerge as effective indicators for ecosystem assessment in the course of acquiring multiple data on ever-changing ecosystems and evaluating the analytical models themselves.

I extracted the two-dimensional PC plane that maximally differentiated the operations A/B/C by choosing a pair of PCi and PCj ($i < j$) that gave the maximum value of S defined as follows:

$$S := \sqrt{(meanA_i - meanB_i)^2 + (meanA_j - meanB_j)^2} \times \sqrt{(meanB_i - meanC_i)^2 + (meanB_j - meanC_j)^2} \times \sqrt{(meanC_i - meanA_i)^2 + (meanC_j - meanA_j)^2},$$

where i and j are the order of PC, and meanAi, meanAj, meanBi, meanBj, meanCi, meanCj are the mean PCi or PCj scores of the sample groups with the A/B/C operations.

The principal components (PC) are linear combinations of the variables and these eigen vectors, on which factor loadings are calculated. The factor loadings correspond to inter-subjective objective measures in MCA [Funabashi 2017b], which contain useful information to characterize the response to the A/B/C operations. I separately performed PCA to the subsets of variables that are common in T and R, as well as those specific to each experiment. I also separated subjective and objective variables and analyzed independently. The variables correspondence between T and R and the subjective/objective property are classified in Table 11. The results are shown in Figure 12 and Figure 13.

The factor loading of a variable to a PC represents the Pearson's product moment correlation coefficient between the PC and the variable, therefore its statistical significance was tested using the test of no correlation. The test was performed using the function cor.test(method="pearson", alternative="two-sided"), and p-values were listed in Appendix 24.

Step 2-2. Ontology analysis of PCA: Classify the variables with significant factor loadings of all PCs according to the ontology of sailboat-type navigation: Variables with significant factor loadings of two PCs selected in Step 2-1 correspond to the effective response to the operations A/B/C; the ones with significant factor loadings of the lower-order PCs with a higher proportion of variance than the operation response represent the baseline drift of the experiment ecosystem and environment; and the ones with significant factor loadings of the higher-order PCs with a lower proportion of variance, after excluding the partial overlap with the baseline drift, signify the marginal fluctuation that does not substantially affect the operation response, in correspondence with Figure 8. Based on the p-values of factor loadings and the order of PC, I classified each variable into three categories: 1) Effective responses to the A/B/C operations; 2) drifts of the baseline environment that appear in lower-order PCs but do not contribute

to the distinction between the operations; and 3) marginal fluctuation in higher-order PCs that do not contain significant information on the operations. Variables with a p-value of 0.05 or less for either of the PCs effective for separating A/B/C operations were classified as 1), smaller PCs with a p-value of 0.05 or less were classified as 2), and none of the above were classified as 3). Thus, 1) corresponds to the motion of the sailboat in Figure 8, 2) to the waves and wind that directly affect the sailboat and move it, and 3) to the fluctuations of the waves and wind in the environment that do not affect the sailboat so much. I used the p-values as a criterion of the selection of generative indices: I considered that the effectiveness of generative indices, which would be continuously renewed through time, does not necessarily depend on the absolute value of statistical significance but relative ranking between them.

For the common variables between T and R, I evaluated this consistency as generative indices [Funabashi and Minami 2021] on the PC plane in Step 2-1, with respect to the statistical significance of the factor loadings: I calculated the maximum information of a variable as -log(minP), where minP represents the minimum p-value of the two factor loadings on the A/B/C-distinctive PC plane. I then calculated the geometric mean of the maximum information and evaluated its significance with respect to the corresponding level of the p-value, as listed in Appendix 26. The results are shown in Appendix 25.

Also, classify the significant factor loadings obtained on the PC planes of Steps 2-1 into the two categories of the consistent and past/novel indices (if generative indices are no longer valid as time progresses, they are considered past indices, and newly validated indices are considered novel indices [Funabashi 2017a][Funabashi and Minami 2021]) according to the commonality and significance of variables in T and R: Common variables with repeatedly significant factor loadings in T and R are judged as consistent indices, while exclusive occurrence of variable with significant factor loading in either of T or R is put as candidates of past or novel indices. Since this study is based on a single season experiment, I cannot yet distinguish between the past and novel indices. The evaluation criteria of the consistent and past/novel indices in terms of statistical significance threshold are explained in Figure 14 and Appendix 26.

Step 3. Regression analysis for the extraction of non-invasive proxies: Analyze statistical dependence between multiple variables as a form of MCA to extract the combinations of non-invasive measures as proxies of invasive measurements. These proxies also represent another type of generative indices in the context of DA, namely the generative proxy. Non-invasive variables are subjective plant diversity measures and a

part of objective measures from liquid samples of soil solution that do not totally destruct soil structure for the measurement. Although I intervened in the soil to sample water using negative pressure, I did not directly disrupt the soil and sampling the soil itself, so I classified it as relatively non-invasive in this case. Invasive variables are those that require direct sampling of soil mass and are disruptive to the ecosystem. The invasiveness on soil for each variable measurement is specified in Table 11. A specific focus of the prediction was set to the invasive objective variables that significantly contributed to the separation of the A/B/C operations in Step 2, i.e. that belong to candidates of past/novel indices and consistent indices, other than the background fluctuation represented as non-significant variables.

The MCA in this step takes the form of regression analysis. I consider the regression model of z on x and y with second-order polynomials, such as $z = a+bx+cy+dxy+ex^2+fy^2$. Here, the addition of the terms x, y, x^2, and y^2 are sensitive to the discordance of the units of measurement between x and y, therefore should be avoided from the model. As a result, essential regression analysis could be simplified to the term with the product xy, such as $B(Z) = a + bB(X)B(Y)$, where X is x or 1/x, Y is y or 1/y, and Z is z or 1/z, in which x and y are non-invasive variables that are subjective or objective, and z is taken from invasive objective variables that are consistent or past/novel indices, as classified in the Appendix 23 ("Variables" sheet) and 24 ("Classification of generative index" sheet). B(X) is the Box-Cox transformation of X. I used the lm() function for the regression analysis. I also took y=1 to represent the single-variable regression model $B(Z) = a + bB(X')$, where X' (= x' or 1/x') is another variable of X, as a single proxy to compare the performance of $B(Z) = a + bB(X)B(Y)$ with respect to the p-value of b and R-squared of the linear regression. Among the 80 variables of the consistent indices (69 subjective and 11 objective) and 93 candidates for the past/novel indices (85 subjective and 13 objective) as classified in Appendix 25, I focused on the 24 invasive objective variables symbolized as z, and analyzed the regression of z on other non-invasive variables x', x, and y (subjective or objective). I investigated all combinations of x' vs. z and xy vs. z, using 108 variables for x'/x/y, and 12 variables of z for Todoroki, as well as 328 variables for x'/x/y, and 14 variables of z for Roppongi, and I examined 1) the difference in p-values of the regression parameter b and 2) the difference in R-squared. Additionally, I performed the Kolmogorov-Smirnov test on the cumulative distributions of these differences, using the ks.test() function. The results of Step 3 are shown in Figure 15, Table 12, and Appendix 27.

The correspondence between the Steps 1/2/3 and the conceptual frameworks of DA and MCA is schematically shown in Figure 11. As defined in Funabashi (2017b),

the inter-subjective objectivity is the significance of the correlation between subjective and objective measures (oval area in orange gradient in Figure 11), which is evaluated with the factor loadings on the PC plane in Step 2 and the p-value and R-squared of the regression analysis in Step 3.

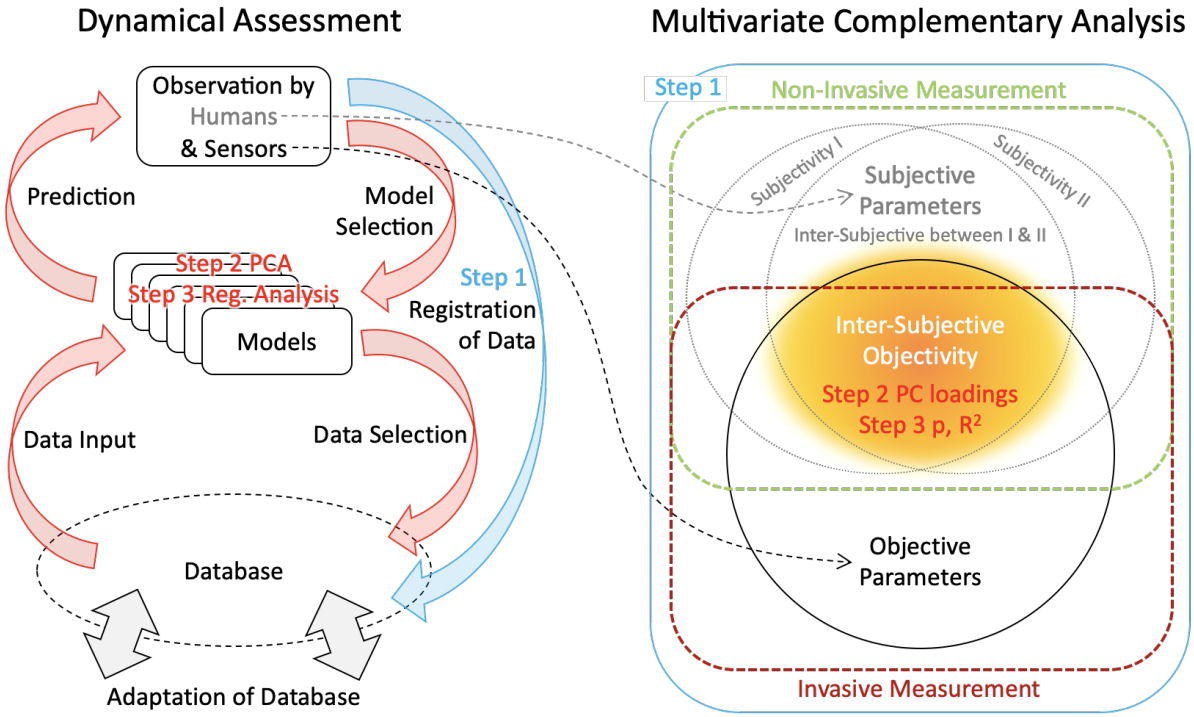


Figure 11. Correspondence between DA-MCA and the analytical steps.
Left: The iterative process of dynamical assessment (DA) according to Funabashi (2017a). Step 1 (blue arrow) corresponds to the registration of data from the experiment T and R. Steps 2 and 3 correspond to the initial cycles of DA with data input, prediction, selection of model and data (red arrows), based on the PCA and regression analysis.
Right: Classification of databases and analytical steps with the conceptual framework of the multivariate complementary analysis (MCA). The MCA integrates subjective human observations (depicting the simplest case with two sets of variables obtained from subjectivity I and II, with gray dotted circles) and objective sensor measurements (black circle), all obtained at the initial Step 1 (blue rounded rectangle). The inter-subjective objectivity that represents the commonality between subjective and objective measures (oval area in orange gradient), is evaluated with the factor loadings on the PC plane in Step 2 and the p-value and R-squared of the regression analysis in Step 3. In general, both subjective and

objective variables can be classified as invasive (inside of a rounded rectangle with a brown dashed line) and non-invasive (inside of a rounded rectangle with a green dashed line) according to the degree of environmental disruption associated with the measurement. In this study, all subjective variables are measured in non-invasive ways and belong to an inter-subjective domain supported by more than two human observers. The objective variables are obtained from both invasive and non-invasive measurements, as specified in Table 11. In Step 3, I consider the non-invasive variables as the proxies of invasive measures, which would lead to exploring the database adaptation in DA.

4-3 Results and Discussion

I obtained in total 350 variables including the variants in Step 1, and the results of Step 2-1 were summarized in Figure 12, Figure 13, and Appendix 24. Figure 12 and 13 show the PCA plots with all/subjective/objective variables in Todoroki and Roppongi on the most distinctive PC planes that distinguish between A/B/C operations (see step 2-1 in Section 4-2). The directions of the three arrows represent the mean PC scores of the sample groups with A/B/C operations, and they were distinctively separated for the selected PC combinations in Figure 12 and 13. Most of the distinctive PC planes were detected in PC1-4, except the case of objective variables in Roppongi that was distinctive on PC12 and PC15 (Figure 12 right bottom). This indicates that the responses of the objective variables to the A/B/C operation in Roppongi were relatively smaller than the baseline drift existing in the environment.

Subjective plant diversity and its composition ratio dominantly capture the different responses to the A/B/C operations, which shows the effectiveness of the operations and reproducibility of responses on aboveground biodiversity. In addition, objective soil variables also reflect the correlated effects with the A/B/C operations when analyzed independently, such as microbial diversity/soil three-phase ratio/permeability in Todoroki (T) and the spatial variability of soil hardness and temporal variation in soil hardness/pH/CEC in Roppongi (R), which provide anchor data that support the objectivity of total measurement. The database of each experiment T and R, as well as the common variables between T and R, were successfully classified (see Appendix 25) into three categories (effective responses to the A/B/C operations; drifts of the baseline environment; and marginal fluctuation), indicating that the measurements were sufficiently diverse to be evaluated as the sailboat-type navigation. The difference between T and R in its baseline variability was particularly enhanced in the PC plane of objective variables (Figure 12 right, top and bottom): distinctive responses to A/B/C

operations do exist on the PC12 - PC15 plane, but lower-order PCs reflect the relatively large variance of many variables compared to the effective response (RoppongiObjectivePCA sheet in Appendix 25). Nevertheless, this baseline drift can be largely suppressed, and the operation responses appear in PC2 - PC5, if I perform PCA using only mutual objective variables in T and R (Figure 13 bottom right). This means that the operation response can be buried if many variables that strongly reflect baseline drift are obtained. Therefore, the analysis should not be easily interpreted as meaningless because the PC dimension has a large value, but should be conducted with this in mind (i.e., data and model selection in Figure 11).

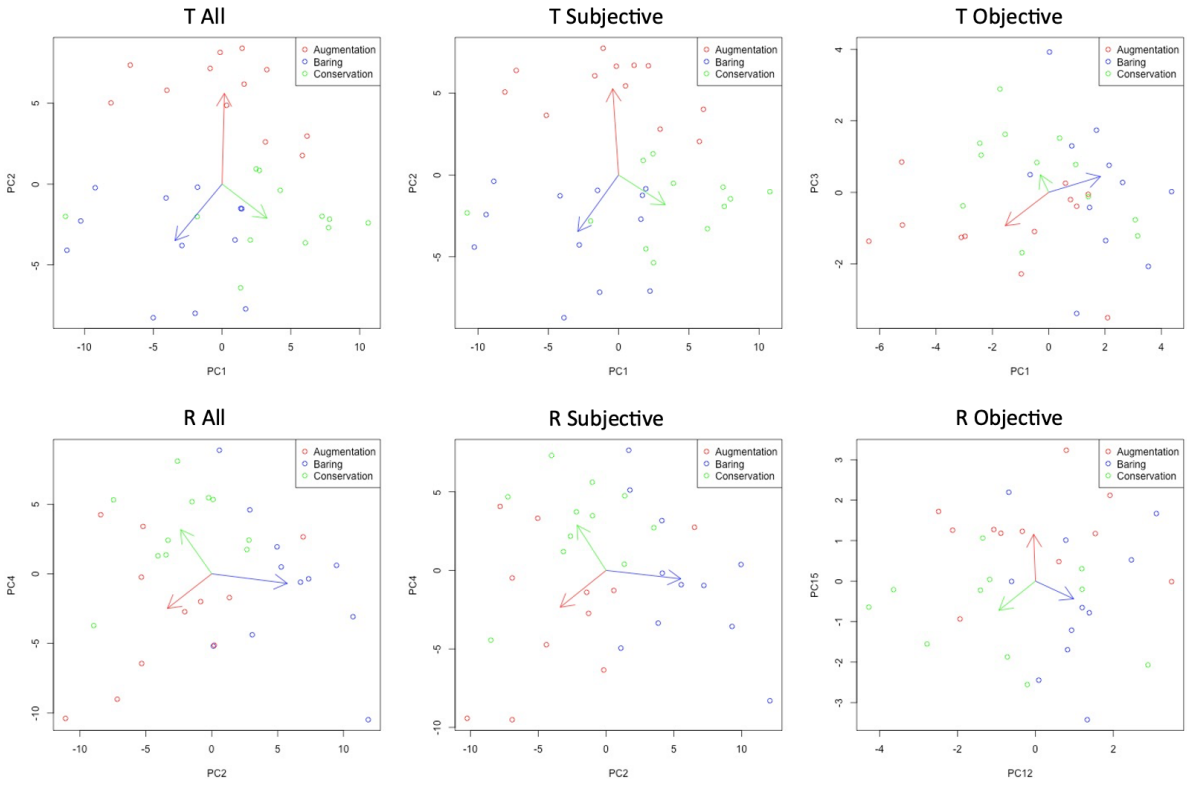


Figure 12. Two-dimensional PC planes that maximally differentiate the operations A/B/C on the sets of all/subjective/objective variables in each experiment T and R.
Left column: Results on all variables in T(top) and R(bottom).
Middle column: Results on the subjective variables of plants in T(top) and R(bottom).
Right column: Results on the objective variables of soil in T(top) and R(bottom).

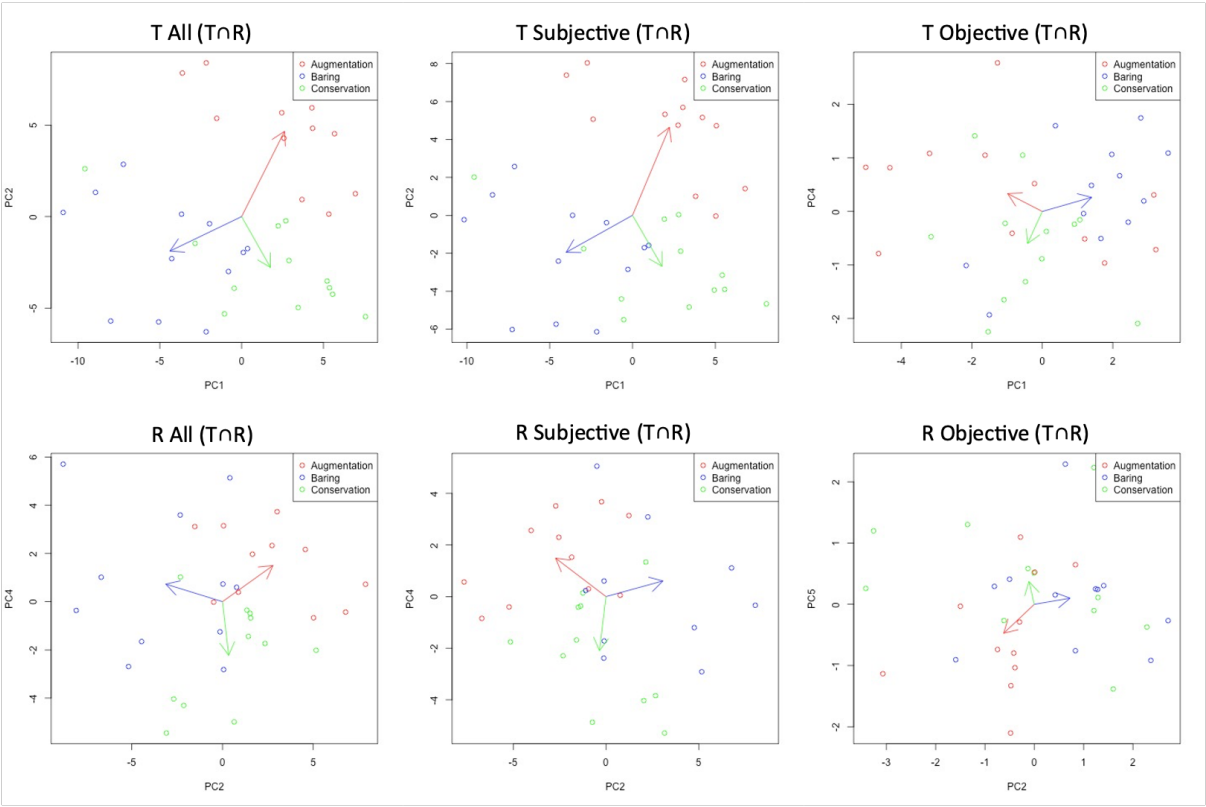


Figure 13. Two-dimensional PC planes that maximally differentiate the operations A/B/C on the sets of all/subjective/objective variables that are common in the experiments T and R.
Left column: Results of T(top) and R(bottom) on all common variables in T and R.
Middle column: Results of T(top) and R(bottom) on the common subjective variables of plants in T and R.
Right column: Results of T(top) and R(bottom) on the common objective variables of soil in T and R.

The ontology analysis in Step 2-2 is summarized in Figure 14 and Appendix 26. I assumed that if the same A/B/C operation was performed in several open environments and qualitatively similar changes corresponding to each operation were observed in all situations, there should be more plausibility that these changes were caused by the operation rather than environment-specific drifts or fluctuations. Among the 86 common variables between T and R, 68 were found to be significant in terms of the consistency level defined by the product of maximum information with the p-value threshold 0.05 (PCA ALL sheet in Appendix 26). Besides the subjective plant diversity measures sensitive to the A/B/C operations such as the plant composition ratio, objective physical parameters such as soil hardness and liquid-phase ratio were revealed to

be valid as consistent indices (see Figure 14 left T∩R All). The consistency of subjective indices is well preserved in the analysis limited to the subjective variables (Figure 14 middle T∩R Subjective). Besides, selective analysis on the objective variables further revealed the consistent validity of soil measures such as water permeability (SHCBC) and microbial diversity and activity (SMDVV) (Figure 14 right T∩R Objective).

As for the variables exclusively specific to T or R, it requires another experiment to judge their consistency. These variables can be provisionary interpreted as the candidates for the "past" or "novel" generative indices according to the future reproducibility [Funabashi 2017a]: If the variable increases its significance on the PC plane of operation response in other experiments, it will be considered as a novel index and be incorporated in a part of consistent indices. While if the variable becomes significant only to interpret a limited number of experiments in a given period, it will be set aside as a part of the previously valid past indices. For example, the amount of humus before the experiment period is clearly the detection of preexisting bias despite the randomized A/B/C allocations in R (See Appendix 26 "PCA Objective" sheet). Among the 264 mutually exclusive variables (22 for T＼R and 242 for R＼T), 12 and 73 subjective variables as well as 7 and 6 objective variables of T and R, respectively, were judged as the candidates for the past/novel indices with respect to the p-value threshold 0.05. The candidates comprised chemical features of soil typically used in agronomy, such as CEC and total carbon and nitrogen.

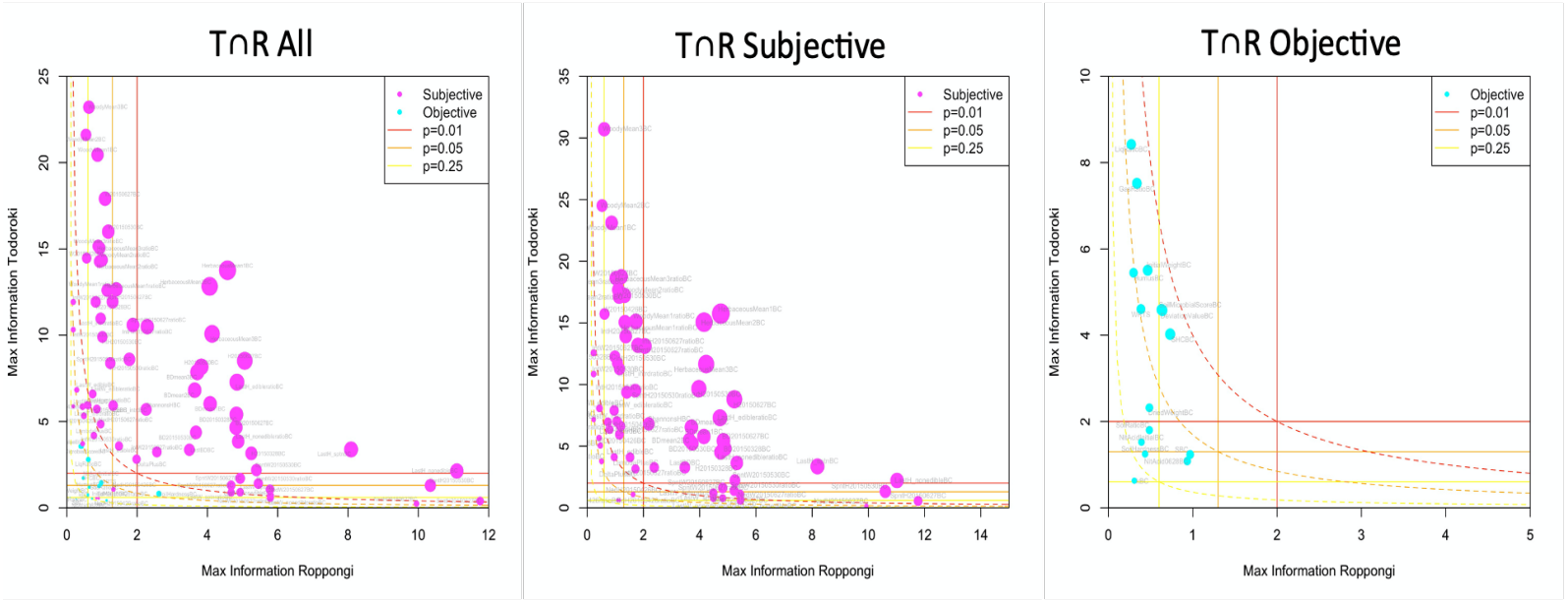


Figure 14. Maximum information of the common variables in T and R on the PC plane of Figure 15.
Left: Maximum information of the factor loadings of the variables responsive to the A/B/C operations in the experiment T(Y-axis) and R(X-axis) on all common variables in T and R.
Middle: Results on the common subjective variables of plants in T and R.
Right: Results on the common objective variables of soil in T and R.

The red/orange/yellow solid lines represent the threshold p-values 0.01/0.05/0.25, respectively, and the dashed lines correspond to the functions Y-axis = Ip^2 / X-axis, where Ip^2 is the squared product of the information -log(threshold p-value). The gray labels in the figures show the variable names as specified in Appendix 23. The higher and righter of the figure, the stronger the indicator has been changed by the ABC operation in both T and R. Higher resolution version is available in Appendix 28.

The results of Step 3 are shown in Figure 15. Compared to the regression with only x', both the p-value information and R-squared increased with the regression on xy: in total the mean difference ± standard deviation of 0.758 ± 0.376 (Todoroki) and 1.067 ± 0.299 (Roppongi) were detected as the decreases in p-values measured as the increases in its information; and 0.0680 ± 0.0346 (Todoroki) and 0.1050 ± 0.0231 (Roppongi) increases in R-squared. These differences correspond to the statistical significance of less than 5% threshold in the Kolmogorov-Smirnov test, as detailed in Table 12. It represents an increase of information that could be utilized to obtain better non-invasive proxies of z (e.g. from the brown line "T x vs z" to green line "T xy vs Z" in Figure 15).

The list of invasive generative indices z and the best regression with non-invasive x' and xy is given in Appendix 27. Notable examples common in T and R are the regression of microbial diversity and vitality values (SMDVV) and its deviation scores on multiple subjective plant diversity measures and nitric acid. Physical properties of soil such as hardness and water retention, as well as chemical properties such as CEC, total carbon (C), and total nitrogen (N) could also find better proxies xy mainly comprised of the subjective plant diversity variables. These proxies are ecologically reasonable as the aboveground plant diversity can affect soil physical property through the extension of the root system, as well as the interaction with underground microbiota that influence soil chemical property and organic matter composition.

The results imply there exists untapped potential of non-linear relationships in non-invasive variables, especially for subjective biodiversity measures, that could predict an important part of the invasive objective measurements through non-linear regression analysis. It is noteworthy that the analysis in Step 3 investigated exhaustive combinations between non-invasive subjective and objective variables, which resulted in the dominance of subjective variables as best proxies. Indeed, human subjective grading of ecosystem (Grading of Ecosystem) alone was collectively correlated with soil mineral and ion parameters such as electrical conductivity, cation exchange capacity, ammoniacal nitrogen, nitrate nitrogen, exchangeable lime, exchangeable potassium, with the statistical significance level less than 5 % in Roppongi (see Appendix 29). Further engagement and training of human ingenuity to grasp diverse ecological situations would be fruitful to extend effective databases and models. It should also be noted that the subjective plant diversity measures in this study could be combined and/or replaced with objective measurement of species diversity such as DNA barcoding (e.g. Boldsystems).

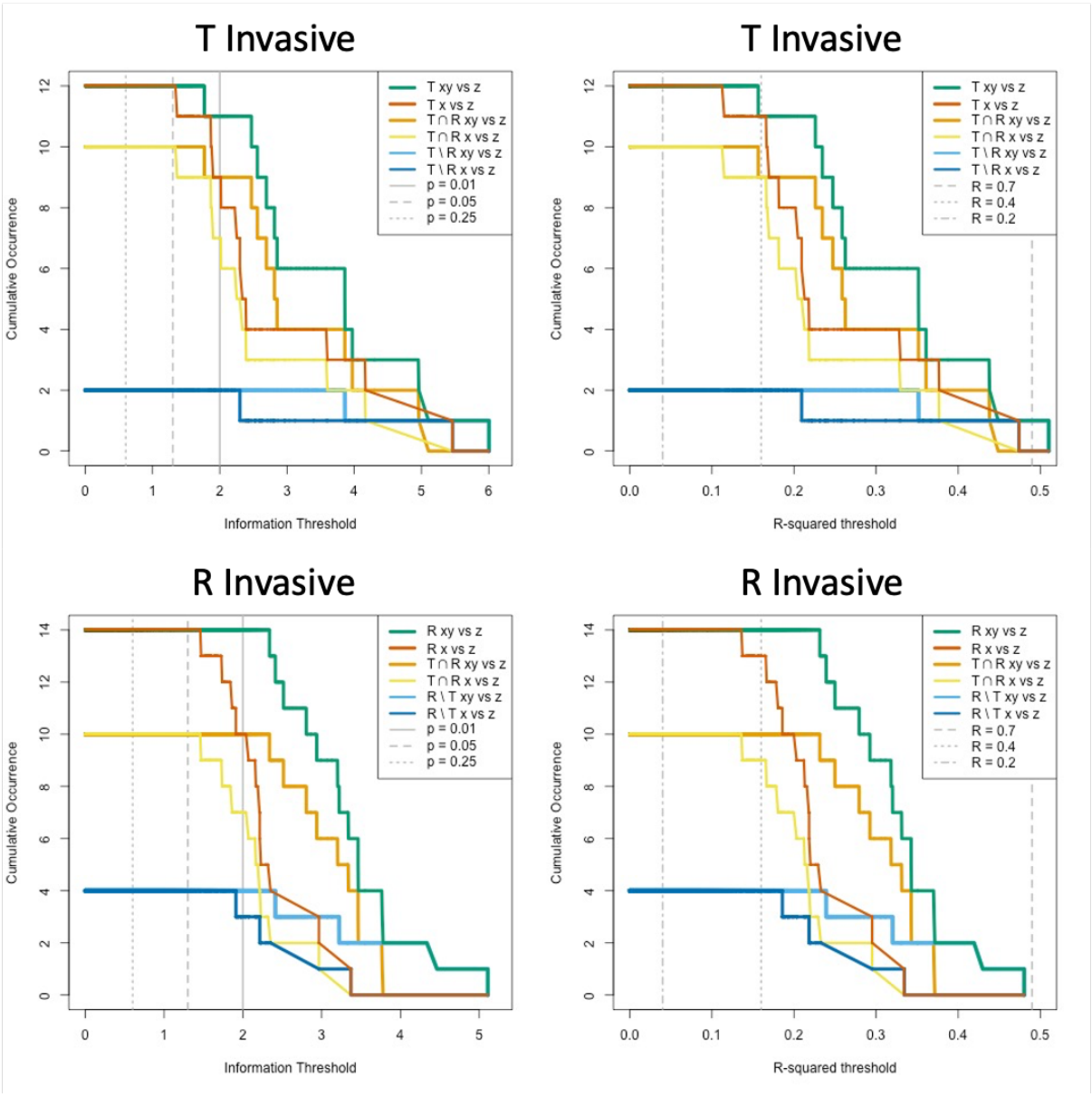


Figure 15. Cumulative occurrence of invasive generative indices with respect to the significance and goodness-of-fit thresholds of linear regression with non-invasive variables.

The horizontal difference between the lines x′ vs. z (i.e. regression model z=a+bx′, with parameters a and b) and xy vs. z (z=a+bxy) represents the amelioration as a proxy of z.

Left: Results of the regression analysis of z on x′ and xy in the experiment T(top) and R(bottom). The vertical axis counts the number of invasive generative indices z that have smaller p-values of the regression coefficient b on any of non-invasive variables x′ or xy, compared to the threshold p-value that is represented as the

information -log(p-value) in the horizontal axis. Therefore the information threshold in the horizontal axis represents the lower bound to count the cumulative occurrence of different z in the vertical axis.

Right: Cumulative occurrence of invasive generative indices z, with respect to the lower bound threshold of R-squared error of the regression analysis on x′ and xy in the experiment T(top) and R(bottom).

Legend: Line colors of the cumulative occurrence differ according to the subset of variables, in which T∪R represents all variables of T and R, T∩R the common variables in T and R, and T⧹R and R⧹T are the ones exclusive to T and R, respectively. For visibility, x′ is denoted by x in the legend.

Table 12. Differences in the information and R-squared of the best regression models in Figure 15.

For each subset of variables, the mean ± standard deviation of the differences in the p-value information and R-squared between the two regression models x′ vs. z and xy vs. z are listed, as well as the p-values of the Kolmogorov-Smirnov test on these differences.

	Information = -log(p-value)			R-squared		
	Mean Difference	Standard Deviation	P-value of KS test	Mean Difference	Standard Deviation	P-value of KS test
Todoroki T∩R	0.69882849	0.30322351	0.05245	0.06369841	0.02675676	0.05245
Todoroki T⧹R	1.0513531	0.72028038	1	0.0893203	0.0750146	1
Todoroki T	0.75758259	0.37579334	0.03144	0.06796872	0.03459459	0.03144
Roppongi T∩R	0.971280695	0.166356161	0.002057	0.098284638	0.01691512	0.002057
Roppongi R⧹T	1.306839323	0.679026	0.2286	0.121907419	0.057355906	0.2286
Roppongi R	1.067154589	0.299497024	0.001021	0.105034004	0.023100062	0.001021

Although the measurements in T and R comprise a large number of variables, these experiments are not yet sufficiently comprehensive to separate the possible dependency of the data on environmental particularity. For example, the dependency of the data on the location and/or the methods of measurement in the experiment T cannot be separated from the seasonal effects that could happen during the experimental period of April-June. Similarly, the locational and methodological particularity in the

experiment R may be mixed with the seasonal effects in October-January. Such problems can be addressed by expanding the database over a longer period and introducing the criteria of multicollinearity analysis.

The analyses in this chapter are based on the linear combination of the variables in Step 2, and partial extension to the second-order products of the variables in Step3. Still, a wider range of non-linear relationships possibly inherent in the ecosystems remains unclear, which could be profoundly addressed by taking machine learning approaches such as deep learning neural networks that possess a vast capacity for classification with non-linear feature values [Funabashi and Minami 2021][Funabashi 2017b].

4-4 Conclusion

I established two independent augmented ecosystems in urban environments, and performed three different operations A/B/C on plant diversity. Despite the presence of large environmental drifts, analysis of the effective responses to the biodiversity operations led to the extraction of the components involved in the actual changes, including the soil variables other than plant diversity. The obtained results of PC planes and these contributing variables could be interpreted as the consistent structure of ecological responses against the steering wheel of the ABC navigation model. Furthermore, the generation proxy analysis showed that a combination of non-invasive measurements can increase the accuracy of estimating invasive soil variables, which could lead to the refinement towards more cost-efficient and less disruptive methods.

The results provided validation on the first steps of navigation in an open and complex ecosystem. By setting up small monitoring spots (e.g., [Fukuda et al. 2020]) and performing A/B/C operations, the DA-MCA framework can extract PC planes that clearly sense response consistency and site specificity. In this study, I focused on ABC operations, which are fundamental in Synecoculture. However, if artificial operations on ecosystems (fertilizer application, tree felling, etc.) and associated data are accumulated, they may able to support the selection of ideal operations to achieve goals in a variety of methods not limited to Synecoculture.

5: General Discussion

One of the objectives of this study was to examine one aspect of Synecoculture that is believed to contribute to solving the trilemma by examining the quality of the products of Synecoculture. This point could be verified by fatty acid analysis of arugula and the metabolome of coarse green tea over a period of more than five years. It was suggested that the products of Synecoculture could be in a different metabolic state compared to the products of conventional agriculture and could affect human health. However, a larger study with different variety of products is necessary to generalize the results of this study since the sample size and the origin of products are limited in this study.

Another objective was to test the practical aspect of Synecoculture through a soil analysis and human assessment of Synecoculture. In Chapter 2, statistical comparisons of single components of the tea leaves did not show differences, but the two products could be distinguished by the amount of several components. The sensory evaluation also revealed that the difference in the multiple compounds could be detected to some extent by the human sense of taste (which is also a comprehensive perception of the many components). This is similar with the results of Chapter 3, where the values of soil microbial diversity and activity were qualitatively consistent with the human subjective evaluation of soil. This offers a possibility that in the future, by training people to compare and analyze objective data, it could be possible to estimate the condition of both products and soil for a given item within a certain margin of error, without having to perform numerous expensive objective measurements. Chapter 4 also suggests that multiple non-invasive indicators can increase the accuracy of estimating invasive indicators, indicating the potential for lower-cost ecological assessment as data are accumulated.

Since human subjective evaluation can perceive many variables and their combined states, it is theoretically possible to evaluate complex systems as a whole. In this regard, human evaluation could be used not only in Synecoculture, but also in other farming systems. It is possible to verify whether or not the human subjective evaluation matches the objective data, and repeating this process can be used as ecosystem evaluation training in the future. Further research on the relationship between objective analysis and human cognition and many feedback from the objective analysis to humans could improve human ability to observe nature and reveal new insights.

The shift from simplistic conventional farming methods to environmentally friendly agriculture will inevitably increase biodiversity, regardless of the method, and to effectively manage this ecosystem, many variables must be taken into account to

make the right decisions. Efficient understanding not only of the parts (e.g., the relationship between soil moisture and yield), but also of the whole, consisting of many subsystems, will contribute to the efficient practice of sustainable agriculture.

6: Conclusion

This study focused on food production under *in natura* culture conditions, aspects of Synecoculture, which has been proposed as a solution to the food, environment, and health trilemma, and verified it by comparing its quality with that of conventional farming methods. The results of fatty acid analysis of arugula and metabolome analysis of tea suggested that products under *in natura* conditions can be different from those under *in cultura* conditions. The study also observed that ingestion of products in different states had different effects on the human body in the form of changes in activity level. It is hoped that larger-scale studies will validate the results of this study and further elucidate the changes in plant metabolism and effects on humans that occur under different culture conditions.

I also focused on the aspect of maintaining and managing high biodiversity in Synecoculture. By combining indicators from many objective analyses with human evaluations and accumulating data, the possibility was shown that ecosystems could be evaluated and utilized for management at a lower cost.

It is expected that further research related to Synecoculture from various perspectives will further evaluate the effectiveness of this method, including its effects on products, soil, human health, and society.

7: References

Papers and Books:

Aho K, Derryberry D, Peterson T. Model selection for ecologists: the worldviews of AIC and BIC, *Ecology* **2014,** 95: 631–636.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in Plant Science* **2019**, 10: 1068.

Bello MGD, Knight R, Gilbert JA, Blaser MJ. Preserving microbial diversity. *Science* **2018**, 362: 33–34.

Box GEP, Cox DR. An analysis of transformations. *Journal of the Royal Statistical Society. Series B*, **1964,** 26: 211-252.

Bredesen DE, Sharlin K, Jenkins D, Okuno M, Youngberg W, Cohen SH, Stefani A, Brown RL, Conger S, Tanio C et al. Reversal of cognitive decline: 100 patients. *Journal of Alzheimers Disease & Parkinsonism* **2018**, 8: 450.

Brunner E, Munzel, U. The nonparametric behrens-fisher problem: asymptotic theory and a small-sample approximation. *Biometrical Journal* **2000**, 42: 17–25.

Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. *Mini-Reviews in Medicinal Chemistry* **2011**, 11: 298-344.

Clemente JC, Pehrsson EC, Blaser MJ, Sandhu K, Gao Z, Wang B, Magris M, Hidalgo G, Contreras M, Noya-Alarcón Ó et al. The microbiome of uncontacted Amerindians. *Science Advances* **2015**, 1: e1500183.

Daley CA, Abbott A, Doyle PS, Nader GA, Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal* **2010,** 9: 10.

de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **1994,** 343: 1454-1459

Defois C, Ratel J, Garrait G, Denis S, Goff OL, Talvas J, Mosoni P, Engel E, Peyret P. Food chemicals disrupt human gut microbiota activity and impact intestinal homeostasis as revealed by in vitro systems. *Scientific Reports* **2018**, 8: 11006.

2030 Douaud G, Refsum H, de Jager CA, Jacoby R, Nichols TE, Smith SM, Smith AD. Pre-
2031 venting Alzheimer's disease-related gray matter atrophy by B-vitamin treatment.
2032 *Proceedings of the National Academy of Sciences in the United States of America* **2013,** 110:
2033 9523–9528.

2034 Fukuda K, Honjo Y, Funabashi M. Synecoculture principles learning kit ver. 0.33e.
2035 Sony Computer Science Laboratories, Inc. and Synecoculture Association **2020,**
2036 https://www.sonycsl.co.jp/wp-content/uploads/2020/10/syne-
2037 cokit_ver033e20201016.pdf

2038 Funabashi M. Foundation of Synecoculture: toward an agriculture of synthetic and
2039 profitable ecosystems. actes du colloque "Transversalités de l'Agriculture Bi-
2040 ologique" **2011,** https://synecoculture.sonycsl.co.jp/CSDC_e-laboratory/Bio-
2041 DevabArticle/Foundation_of_Synecoculture-English_Color.pdf

2042 Funabashi M. Food components as markers linking health and environment: statistical
2043 invariance analysis of in natura diet. *American Journal of Bioscience and Bioengineering*
2044 **2015**, 3: 183.

2045 Funabashi M. editor « Synecoculture Manual 2016 version » (English version). Re-
2046 search and education material of UniTwin UNESCO Complex Systems Digital Cam-
2047 pus, e-laboratory: Open systems exploration for ecosystems leveraging, No.2. **2016a,**
2048 https://synecoculture.sonycsl.co.jp/public/2016%20Synecoculture%20Manual_com-
2049 pressed.pdf

2050 Funabashi M. Synecological farming: Theoretical foundation on biodiversity responses
2051 of plant communities, *Plant Biotechnology* **2016b,** 33: 213-234.

2052 Funabashi M. Open systems exploration: an example with ecosystems management.
2053 *First Complex Systems Digital Campus World E-Conference 2015*, Springer Proceedings
2054 in Complexity **2017a,** 223-243.

2055 Funabashi M. Citizen science and topology of mind: complexity, computation and criti-
2056 cality in data-driven exploration of open complex systems. *Entropy* **2017b,** 19: 181.

2057 Funabashi M. Synecological farming for mainstreaming biodiversity in smallholding
2058 farms and foods: implication for agriculture in India. *Indian Journal of Plant Genetic*
2059 *Resources* **2017c**, 30: 99–114.

2060 Funabashi M. Human augmentation of ecosystems: Objectives for food production and
2061 science by 2045. *NPJ Science of Food* **2018**, *2*: 1–11.

2062 Funabashi M. Augmentation of plant genetic diversity in synecoculture: theory and
2063 practice in temperate and tropical zones in genetic diversity in horticultural plants.
2064 *Series: Sustainable Development and Biodiversity* (ed. Nandwani, D.) Springer **2019,**

2065 Funabashi M. Living in a hotspot of city and biodiversity. the case of Synecoculture. P.
2066 252-253. In: Mejía, M.A., Amaya-Espinel, J.D. (eds.). BiodiverCities by 2030: Trans-
2067 forming Cities with Biodiversity. Bogotá. Instituto de Investigación de Recursos Bio-
2068 lógicos Alexander von Humboldt. **2022,** 288.

2069 Funabashi M, Ohta K. Flavonoid-rich secondary metabolites in naturally grown green
2070 tea are correlated with a higher shift of the consumers' excise level. *Journal of Food*
2071 *Science & Nutrition* **2020**, 6: 63.

2072 Funabashi M, Minami T. Dynamical assessment of aboveground and underground bio-
2073 diversity with supportive AI. *Measurement: Sensors* **2021**, 18: 100167.

2074 Funabashi M, Hanappe P, Isozaki T, Maes A, Sasaki T, Steels L, Yoshida K. Foundation
2075 of CS-DC e-laboratory: OPEN SYSTEMS EXPLORATION FOR ECOSYSTEMS LEV-
2076 ERAGING. In *First Complex Systems Digital Campus World E-Conference 2015*; Bour-
2077 gine P, Collet P, Parrend P, Eds, Springer International Publishing: Cham, Switzer-
2078 land, **2017**, 351–374.

2079 Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. Interindividual variability in
2080 sleeping metabolic rate in Japanese subjects. European jounal of clinical nutrition.
2081 **2007**, 11: 1256-1261.

2082 Golisz A, Lata B, Gawronski SW, Fujii Y. Specific and total activities of the allelochemi-
2083 cals identified in Buckwheat. *Weed Biology and Management* **2007**, 7: 164–171.

2084 Gross RL, Newberne PM. Role of nutrition in immunologic function. *Physiological Re-*
2085 *views* **1980**, 60: 188–302.

2086 Huang J, Wang Y, Xie Z, Zhou Y, Zhang Y, Wan X. The anti-obesity effects of green tea
2087 in human intervention and basic molecular studies. *European Journal of Clinical Nu-*
2088 *trition* **2014**, 68: 1075-1087.

2089 IAASTD. Agriculture at a crossroads: Synthesis report. International Assessment of
2090 Agricultural Knowledge, Science and Technology for Development **2008,**
2091 **h**ttps://wedocs.unep.org/handle/20.500.11822/7862

2092 Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids*
2093 *Research* **2000**, 28: 27–30.

2094 Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A,
2095 Jones KE, Mitchell CE et al. Impacts of biodiversity on the emergence and transmis-
2096 sion of infectious diseases. *Nature* **2010**, 468: 647–652.

2097 Kessner D, Chambers M, Burke R, Agus D, Mallick P. ProteoWizard: Open source soft-
2098 ware for rapid proteomics tools development. *Bioinformatics* **2008**, *24*, 2534–2536.

2099 Lekshmi SSU, Singh DN, Baghini MS. A critical review of soil moisture measurement,
2100 *Measurement* **2014**, 54: 92-105.

2101 Lewis SL, Maslin MA. Defining the anthropocene. *Nature* **2015**, 519: 171-180.

2102 Liu RH. Health benefits of fruit and vegetables are from additive and synergistic com-
2103 binations of phytochemicals. *American Journal of Clinical Nutrition* **2003**, *78*: 517S–
2104 520S.

2105 Middleton EJR, Kandaswami C, Theoharides TC. The effects of plant flavonoids on
2106 mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharma-*
2107 *coogical Reviews* **2000**, 52: 673–751.

2108 Miyashita K, Nara E, Ota T. Oxidative stability of polyunsaturated fatty acids in an
2109 aqueous solution. *Bioscience, Biotechnology and Biochemistry* **1993**, 57: 1638-1640.

2110 Moradzadeh S, Moghaddam SS, Rahimi A, Pourakbar L, El Enshasy HA, Sayyed RZ.
2111 Bio-chemical fertilizer improves the oil yield, fatty acid compositions, and macro-
2112 nutrient contents in nigella sativa l. *Horticulturae* **2021,** 7: 10.

2113 Nishi A, Ohbuchi K, Kushida H, Matsumoto T, Lee K, Kuroki H, Nabeshima S,
2114 Shimobori C, Komokata N, Kanno H et al. Deconstructing the traditional Japanese
2115 medicine "Kampo": compounds, metabolites and pharmacological profile of maoto,
2116 a remedy for flu-like symptoms. *NPJ Systems Biology and Applications* **2017**, 3: 1–11.

2117 Oshima Y, Kawaguchi K, Tanaka S, Ohkawara K, Hikihara Y, Ishikawa-Tanaka K,
2118 Tabata I. Classifying household and locomotive activities using a triaxial accelerom-
2119 eter. *Gait Posture* **2010**, 31: 370-374.

2120 Ohkawara K, Oshima Y, Hikihara Y, Ishikawa-Takata K, Tabata I, Tanaka S. Real-time
2121 estimation of daily physical activity intensity by a triaxial accelerometer and a

gravity-removal classification algorithm. *The British journal of nutrition*. **2011**, 105: 1681-1691.

Papadopoulos AP, Bar-Tal A, Silber A, Saha U, Raviv M. Inorganic and synthetic organic components of soilless culture and potting mixes, in: Raviv M, Lieth JH (Eds.), *Soilless Culture*, Elsevier, **2008**, 505

Pereira C, Li D, Sinclair AJ. The alpha-linolenic acid content of green vegetables commonly available in Australia. *International Journal for Vitamin and Nutrition Research*. **2001**, 71: 223-228.

Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JP, Fernandez-Manjarrés JF, Araújo MB, Balvanera P, Biggs R, Cheung WW et al. Scenarios for global biodiversity in the 21st century. *Science* **2010**, 330: 1496-1501.

Rail LC, Meydani SN. Vitamin B6 and immune competence. *Nutrition Reviews* **1993**, 51: 217–225.

Rice EL. *Allelopathy*; Academic Press, New York, USA, **1974**.

Sakurai N, Ara T, Kanaya S, Nakamura Y, Iijima Y, Enomoto M, Motegi T, Aoki K, Suzuki H, Shibata D. An application of a relational database system for high-throughput prediction of elemental compositions from accurate mass values. *Bioinformatics* **2013**, 29: 290–291.

Sakuramoto N, Yokoyama K, Iekushi T. Advocacy of positive environmental assessment using soil microbial diversity and its vitality value as an index for environmental preservation effects in environmental accounting. *Proceedings of AFITA 2010 International Conference, The Quality Information for Competitive Agricultural Based Production System and Commerce* **2010**, 137-140.

Sánchez-Bayo F and Wyckhuys KAG. Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation* **2019**, 232: 8-27.

Shetty P, Youngberg W. Clinical lifestyle medicine strategies for preventing and reversing memory loss in alzheimer's. *American Journal of Lifestyle Medicine* **2018**, 12: 391–395.

Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy* **2002**, 56: 365–379.

2152 Stark AH, Crawford MA, Reifen R. Update on alpha-linolenic acid. *Nutrition Reviews*
2153 **2008**, 66: 326-332.

2154 Stohs SJ, Badmaev V. A review of natural stimulant and non-stimulant thermogenic
2155 agents. *Phytotherapy Research* **2016**, 30: 732-740.

2156 Takeshita T, Ota S, Yamazaki T, Hirata A, Zachleder V, Kawano S. Starch and lipid ac-
2157 cumulation in eight strains of six Chlorella species under comparatively high light
2158 intensity and aeration culture conditions. *Bioresource Technology* **2014**, 158: 127-134.

2159 Tilman D and Clark M. Global diets link environmental sustainability and human
2160 health. *Nature* **2014**, 515: 518–522.

2161 Tindano A and Funabashi M. Proceedings of the 1st African forum on Synecoculture
2162 (English version). *Research and education material of UniTwin UNESCO Complex Sys-*
2163 *tems Digital Campus, e-laboratory: Open systems exploration for ecosystems leveraging,*
2164 *No.5*. **2017**,

2165 Tokoro M. Open systems science: A challenge to open systems problems. *First Complex*
2166 *Systems Digital Campus World E-Conference 2015*, Springer Proceedings in Complexity
2167 **2017**, 213-221.

2168 Trost SG, McIver KL, Pate RR. Conducting accelerometer-based activity assessments in
2169 field-based research. *Medicine and Science in Sports and Exercise* **2005**, 37: 531-543.

2170 Vardavas CI, Majchrzak D, Wagner KH, Elmadfa I, Kafatos A. Lipid concentrations of
2171 wild edible greens in Crete. *Food Chemistry* **2006**, 99: 822-834.

2172 Vudhivai N, Ali A, Pongpaew P, Changbumrung S, Vorasanta S, Kwanbujan K, Char-
2173 oenlarp P, Migasena P, Schelp FP. Vitamin B1, B2 and B6 status of vegetarians.
2174 *Jounal of the Medical Association of Thailand* **1991**, 74: 465–470.

2175 Yamaguchi T, Sugimura R, Shimajiri J, Suda M, Abe M, Hosokawa M, Miyashita K
2176 Oxidative stability of glyceroglycolipids containing polyunsaturated fatty acids.
2177 *Journal of Oleo Science* **2012**, 61: 505-513.

2178 Yang W, Xu X, Li Y, Wang Y, Li M, Wang Y, Ding X, Chu Z. Rutin-mediated priming
2179 of plant resistance to three bacterial pathogens initiating the early SA signal path-
2180 way. *PLoS ONE* **2016**, 11: e0146910.
2181

Other Information:

Agilent. Available online: https://www.chem-agilent.com/pdf/5989-5200JAJP_LO.pdf (accessed on 24 October 2022).

Boldsystems. Barcode of life data system v4. https://www.boldsystems.org/ (Accessed on 14th April 2022).

CRAN. R ver 4.1.0. CRAN R Project (**2022**) https://cran.r-project.org/ (Accessed on 8th April 2022).

DGC. DGC Technology Inc. Available online: https://dgc.co.jp/ (accessed on 1 December 2022).

Flavonoid Viewer. Available online: http://webs2.kazusa.or.jp/mfsearcher/flavonoidviewer/ (accessed on 29 March 2020).

Human Augmentation of Ecosystems UniTwin UNESCO Complex Systems Digital Campus e-Laboratory. Available online: https://www.human-augmentation-of-ecosystems.net/ (accessed on 1 December 2022).

KEGG. Available online: https://www.genome.jp/kegg/ (accessed on 29 March 2020).

KEGG API. Available online: https://www.kegg.jp/kegg/rest/keggapi.html (accessed on 29 March 2020).

KEGG BRITE. Available online: https://www.genome.jp/kegg/brite.html (accessed on 29 March 2020).

KEGG PATHWAY. Available online: https://www.genome.jp/kegg/pathway.html (accessed on 29 March 2020).

KOMICS. http://www.kazusa.or.jp/komics/ja/home-ja/news-ja/213-powerget-3-5-7.html (accessed on 29 March 2020). (KOMICS(A))

KOMICS. Available online: http://www.kazusa.or.jp/komics/en/tool-en/236-powerget-batch.html (accessed on 29 March 2020). (KOMICS(B))

OMRON Health management software BI-LINK Activity Volume PROFESSIONAL EDITION Ver1.0 HMS-HJA-IC01J. **2008** https://www.healthcare.omron.co.jp/support/download/software/bilink_pro_v1.html

Q Exactive. Available online: https://www.thermofisher.com/order/catalog/product/IQLAAEGAAPFALGMAZR (accessed on 29 March 2020).

Sony CSL. Sony Computer Science Laboratories, Inc. Synecoculture Project Page. Available online: https://www.sonycsl.co.jp/tokyo/407/ (accessed on 1 December 2022).

SynecO. Available online: https://www.syneco.inc/ (accessed on 1 December 2022).

Thermo Fisher Scientific. Available online: https://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Marketing%20Material/MassSpectrometryCollateral/Brochures/BR30135_LTQOrbitrapXL_Brochure.pdf (accessed on 29 March 2020). (Thermo Fisher Scientific (A)]

Thermo Fisher Scientific. Available online: https://assets.thermofisher.com/TFS-Assets/CMD/brochures/BR-70133-LC-Dionex-UltiMate-3000-RSLC-BR70133-EN.pdf (accessed on 29 March 2020). (Thermo Fisher Scientific (B))

8: Publications

A: Kousaku Ohta, Tsuyoshi Takeshita, Masatoshi Funabashi & Shoji Oda. *Naturally grown rucola, Eruca sativa, contains more α-linolenic acid than conventionally grown rucola*. Plant Biotechnology 33(4), 2016, Pages 277-279

B: Masatoshi Funabashi & Kousaku Ohta. *Flavonoid-Rich Secondary Metabolites in Naturally Grown Green Tea are Correlated with a Higher Shift of the Consumers' Excise Level*. Journal of Food Science & Nutrition 6: 063. 2020

C: Kousaku Ohta, Tatsuya Kawaoka & Masatoshi Funabashi. *Secondary Metabolite Differences between Naturally Grown and Conventional Coarse Green Tea*. Agriculture 10(12), 2020, 632

D: Kousaku Ohta & Masatoshi Funabashi. *Complementary analyses of soil microbial and chemical properties and human observation on augmented ecosystems in urban environment*. Measurement: Sensors, 18, December 2021, 100333

E: Kousaku Ohta, Godai Suzuki, Kae Miyazawa & Masatoshi Funabashi. *Open Systems Navigation based on System-Level Difference Analysis - Case Studies with Urban Augmented Ecosystems*. Measurement: Sensors, 23, October 2022, 100401

<Conference>

F: Kousaku Ohta, Tanoy Debnath & Masatoshi Funabashi. (Poster) *Sensory evaluation for the distinction between in natura and in cultura culture conditions of coarse green tea*. Sense Asia 2021, 5-7 December 2021 (Online)

G: Kousaku Ohta & Masatoshi Funabashi. (Poster) *Feeding 9 billion People with a Novel Form of Edible Ecosystems Augmented by Humans*. Nature's 150th anniversary symposium THE FUTURE OF JAPANESE SCIENCE, 4 April 2019 (The University of Tokyo, Tokyo, Japan)

A corresponds to Section 2-2, B to Section 2-5, C to Section 2-3, D to Chapter 3, E to Chapter 4, and F to Section 2-4. G is a poster introducing the activities and prospects of Synecoculture.

9: Appendix

Appendix 1. Synecoculture Field in Kashiwa City.

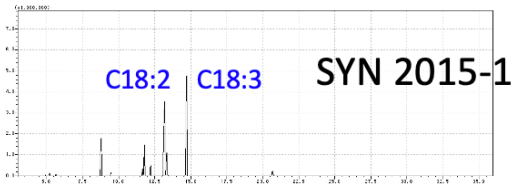
18th May 2015

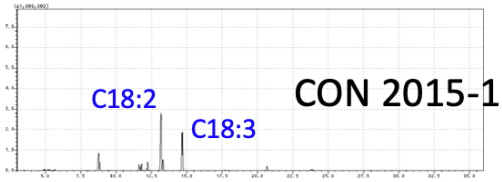
Mixed dense polyculture with various plants

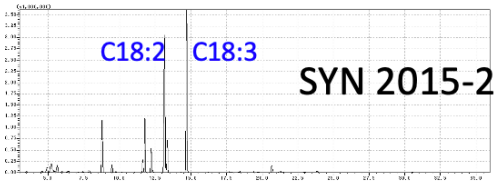
10th Nov. 2014
Basil, mint, and Kudzu

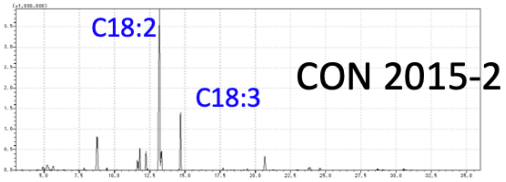
10th Dec. 2015
Arugula and weeds

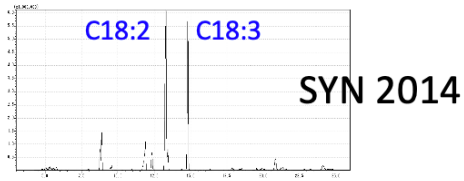
Appendix 2. Chromatograms of arugula samples.

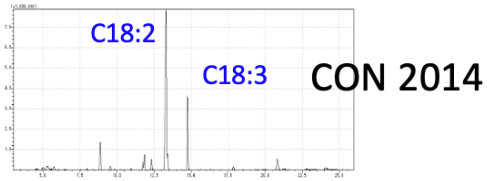


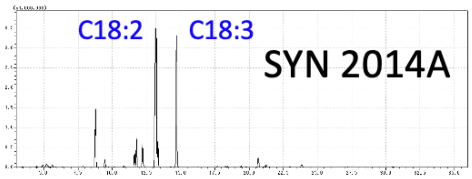












The vertical axis represents the intensity of compounds and the horizontal axis represents the retention time.

Appendix 3. Parameters of LC-MS analysis 2014-2017 and 2018-2019.

Table 13. 2014-2017 Parameters.

Measuring Instrument, Parameter	Model Number, Setting
HPLC	Agilent 1200 series
Column	TSKgel ODS-100V 5 mm 3 x 50 mm(TOSOH)
Column Temperature	40℃
Eluent	Solvent A: Water, B: Acetonitrile, both HPLC grade and added with 0.1% v/v formic acid (all the solvents obtained from FUJIFILM Wako Pure Chemical Corporation)
Gradient Condition (Elapsed Time(min):B%)	0min:3%, 15min:97%, 20min:97%, 20.1min:3%, 25min:3%
Flow Velocity	0.4 mL/min
Injection Volume	5μL
High-Resolution Mass Spectrometer	LTQ ORBITRAP XL(Thermo fisher scientific)
Ionization Method	ESI positive mode
Mass Range	100-1500 m/z
Scan Events (3sec interval for the whole process from event 1 to 5)	Event 1: Full scan with ORBITRAP Event 2: MS/MS measurement by ion trap for ions with the strongest intensities in full scan Event 3: MS/MS measurement by ion trap for ions with the 2nd strongest intensities in full scan Event 4: MS/MS measurement by ion trap for ions with the 3rd strongest intensities in full scan Event 5: MS/MS measurement by ion trap for ions with the 4th strongest intensities in full scan
Photo Diode Array Measurement Range	190-950nm

2278 Table 14. 2018-2019 Parameters.

Ultimate 3000 Analysis conditions	
HPLC	Ultimate 3000 RSLC
Column	InertSustain AQ-C18 (2.1 x 150 mm, 3 mm-particle, GL Science)
Column Temperature	40℃
Mobile Phase	Solvent A: Water, B: Acetonitrile, both HPLC grade and added with 0.1% v/v formic acid (all the solvent obtained from FUJIFILM Wako Pure Chemical Corporation)
Flow Velocity	0.2 ml/min
Injection Volume	2 ml

LC Gradient Program		
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	98	2
3	98	2
30	2	98
35	2	98
35.1	98	2
40	98	2

Q Exactive Analysis conditions	
Measuring Time	3 - 30 min
Ionization Method	Electro Spray Ionization (ESI)
Mass Range	m/z: 80 - 1,200
Full Scan Resolution	70,000
MS/MS Scan Resolution	17,500
MS/MS Precursor	Data Dependent Scan
Dynamic Exclusion	20 sec

Appendix 4 – 29 are available on following URL:
https://drive.google.com/drive/folders/1I4tGVZP6YPk_kBayXen6I4gGD8BmMjvJ?usp=sharing

Appendix 4: Integrated metabolome data matched with KEGG database
Appendix 5: Integrated metabolome data matched with Flavonoid Viewer
Appendix 6: Importance and Cumulative Proportion of the Components of PCA with 2014-2019 samples and other PC plots
Appendix 7: PCA plot of 2014-2017 samples and 2018-2019 samples
Appendix 8: All structural isomers of the 130 distinctive loadings projected onto the "map01110 Biosynthesis of secondary metabolites" of KEGG PATHWAY
Appendix 9: Summary of the statistical distribution of metabolome data
Appendix 10: Negative Loadings of PC3 categorized in KEGG PATHWAY and BRITE
Appendix 11: Positive Loadings of PC3 categorized in KEGG PATHWAY and BRITE
Appendix 12: KEGG BRITE compound classification in the other hierarchy of BRITE
Appendix 13: List of chemical formulae with Syneco-distinctive loadings that can completely separate Syneco and Conv samples in 2014-2017 samples
Appendix 14: List of chemical formulae with Conv-distinctive loadings that can completely separate Syneco and Conv samples in 2014-2017 samples
Appendix 15: List of chemical formulae with Syneco-distinctive loadings that can completely separate Syneco and Conv samples in 2018-2019 samples
Appendix 16: List of chemical formulae with Conv-distinctive loadings that can completely separate Syneco and Conv samples in 2018-2019 samples
Appendix 17: List of chemical formulae with Syneco-distinctive loadings that can completely separate Syneco and Conv samples in 2014-2019 samples
Appendix 18: List of chemical formulae with Conv-distinctive loadings that can completely separate Syneco and Conv samples in 2014-2019 samples
Appendix 19: All structural isomers of the Syneco-distinctive loadings projected onto the "map01120 Microbial metabolism in diverse environments" of KEGG PATHWAY
Appendix 20: All structural isomers of the Conv-distinctive loadings projected onto the "map01120 Microbial metabolism in diverse environments" of KEGG PATHWAY
Appendix 21: Daily results of human physical activity measurement
Appendix 22: All Soil Data of Section 3-3
Appendix 23: All variables and variants measured in experiments T and R
Appendix 24: List of p-values of the pearson's correlation coefficient of the of the Step 2-1
Appendix 25: Results of Step 2-2 Ontology analysis

Appendix 26: Classification Results of Step 2-2 Ontology analysis drawn in Figure 14

Appendix 27: Results of Step 3

Appendix 28: High resolution version of Figure 14

Appendix 29: Results of Experiment R before