이학박사 학위논문

감마 방사선 강도에 따른 미생물군집 변화와 기능유전자 반응

Soil Microbial Community and Functional Gene Response to Gamma Irradiation

2019 년 2 월

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by

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Under the supervision of

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감마 방사선 강도에 따른 미생물군집 변화와 기능유전자 반응

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이 논문을 이학박사 학위논문으로 제출함

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매튜의 이학박사 학위논문을 인준함

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Abstract

Ionizing radiation is a unique pollutant that requires novel ecological approaches and concepts to outline the resulting environmental outcome. However, little is known of the effects of ionizing radiation exposure on soil biota. Here, the soil was exposed to weekly 24-hour bursts of ^{60}Co gamma radiation over a six-week period, at three levels of exposure (0.1 kGy/hr [low], 1 kGy/hr [medium] and 3 kGy/hr [high]). In the first study, soil DNA was extracted and shotgun metagenomes were sequenced and characterised using MG-RAST. It was hypothesized that with increasing radiation exposure there would be a decrease in both taxonomic and functional diversity. While bacterial diversity decreased, the diversity of fungi and algae unexpectedly increased, perhaps because of release from competition. Despite the decrease in diversity of bacteria and of biota overall, functional gene diversity of algae, bacteria, fungi and total biota increased. Thus, cycles of radiation exposure may increase the range of gene functional strategies viable in the soil, which is a novel ecological example of the effects of stressors or disturbance events promoting some aspects of diversity. Repeated density-independent population crashes followed by population expansion may allow lottery effects, promoting coexistence. Radiation exposure produced large overall changes in community composition.

The study suggests several novel radiation tolerant groups: in addition to Deinococcus-Thermus, which reached up to 20 % relative abundance, the phyla Chloroflexi (bacteria), Basidiomycota and Chytridiomycota (fungi) and Nanoarcheota (archaea) are suggested to include radiation-tolerant members. In addition, virus and

transposon abundance increased, perhaps owing to reduced resistance by radiation-stressed cells. Unexpectedly, the relative abundance of 'stress'-related genes decreased at higher radiation doses such as heat shock, detoxification, acid stress and cold shock related genes, but the diversity of dormancy (like persister cells, spore core dehydration, spore germination and sporulation cluster related genes) and DNA-repairs-related genes increased – as might be expected for selection for DNA repair mechanisms.

In the second study, attention was focussed on the ecology of *Deinococcus* – a genus of soil bacteria known for their radiation resistance, in the context of gamma radiation. The soil DNA was extracted following six weekly cycles of irradiated and studied using 16S rRNA amplicon data, annotated metagenome data and published whole genome for *Deinococcus*, to investigate the following questions: 1) How does the bacterial community structure change with increasing radiation exposure, and do different *Deinococcus* species dominate at different radiation intensities – suggesting the existence of 'radiation niches'? 2) What features of the genomes of the *Deinococcus* species that predominate at higher radiation intensities confer greater 'success' at high radiation exposures? 3) What are the overall trophic features of the *Deinococcus* assemblage in radiation-exposed soils, and what might this indicate about ecosystem processes in the irradiated soil?

It was observed that 1) increasing radiation dose produced a major increase in relative abundance of *Deinococcus*, which reached ~80 % of reads at the highest doses. Differing relative abundances of the various *Deinococcus* species with

exposure levels indicate distinct ‘radiation niches’. At 3 kGy/hr, a single OTU identified as D. *ficus* overwhelmingly dominated the mesocosms. 2) Corresponding published genome data show that the dominant species at 3 kGy/hr, *D. ficus*, has a larger and more complex genome than other *Deinococcus* species with a greater proportion of genes related to DNA and nucleotide metabolism, cell wall, membrane and envelope biogenesis as well as more cell cycle control, cell division and chromosome partitioning related genes. It also has a higher guanine-cytosine ratio than most other *Deinococcus*. These features may be linked to genome stability, and explain its greater abundance in this apparently competitive system, under high radiation exposures. 3) Published genome analysis suggests that *Deinococcus*, including *D. ficus,* are capable of utilizing diverse carbon sources derived both from microbial cells killed by the radiation (including C5-C12 containing compounds like arabinose, lactose, N-acetyl-D-glucosamine) and plant-derived organic matter in the soil (e.g. cellulose and hemicellulose). 4) Overall, from its metagenome, even the most highly irradiated (3 kGy/hr) soil possesses a wide range of activities necessary for a functional soil system, such as lignin degradation, P solubilisation and N fixation – although at low abundances. Future studies may consider the resilience and sustainability of such soils in a high radiation environment. In addition to exploring, the extent to which species of *Deinococcus* dominate depending upon the soil type and the differences this might make to the soil functions.

The experimental frameworks of these studies enabled an assessment of functional gene and taxonomic composition of a gamma-irradiated soil community.

These studies demonstrated the capacity of soil microbial community to remain viable under differing doses of ionizing gamma irradiation. This consideration is vital in view of the widespread application of gamma radiation to sterilize soil and other important materials, understanding contaminated environments, potential space travel and the viability of Earth's lifeforms on other planets. To elaborate on these findings, future studies are anticipated to adopt continuous irradiation of soil as opposed to the repeated burst adopted for the present studies, include samples from actual irradiation contaminated sites and incorporate other next-generation sequencing techniques. Such studies will have greater implications for bioremediation, policy formulation, space exploration and harvesting of useful organisms, genes and bioactive compounds from irradiated soils.

Keywords: ^{60}Co Gamma Radiation, Deinococcus-Thermus, Edaphic dysbiosis, Environmental Change, Legacy Effects, Microbiome, Soil Contamination, Radiation Resistance, Radioecology

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PART 1.

ENUMERATING SOIL MICROBIOTA RESPONSE TO GAMMA IRRADIATION USING ADVANCED SEQUENCING TECHNIQUES: AN INTRODUCTION

1.1. Soil Contamination by Ionizing Radionuclide: Radioecology

Ionizing radionuclides are of great practical use (WHO, 2016) but also represents a potential danger in the form of indiscriminate use and disposal (Gbadebo et al., 2012). The environment is not sterile and in most cases like the soil, abound with huge undiscovered microbial life. As a result, when these environments, and the microbial processes that sustain them are exposed to significant radiation doses, the consequences are unpredictable because absorbed doses and rates are difficult to predict since they are mostly heterogeneous and dependent on the activity of the radiation type and decay dynamics (Brown et al., 2015). Therefore, the field of radioecology evolved to foster a general understanding of the impacts of radionuclides to the biotic and abiotic components of the environment.

1.1.1. The Evolving Field of Radioecology

Radioecology addresses the effects of radionuclides on the ecosystem using an interdisciplinary approach to evaluate the benefits and risks associated with their formation, occurrence, behaviour and distribution on key environmental interactions. It adopts applied ecological concepts in an attempt to explain dose-dependent consequences on different ecosystem sustaining mechanisms through designed field studies, laboratory experiments, monitoring and impact assessment, or predictive simulation models.

Radioecology began in the mid-twentieth century when ecologists sought to understand the evolution and environmental effects of radionuclides concentrations in ecosystems. It coincided with the discovery of X-rays by W.K. Roentgen, the phenomena of radioactivity by A. Becquerel and radioactive elements by M. Curie (Alexakhin, 2006) although they were not interested in potential ecological effects. The field became popular much later in the 1950's and early 1960's due to increased nuclear weapon testing, nuclear power, public awareness, the application of radionuclides in nuclear weapon development, testing and electric power generation, accidents at nuclear facilities, management of naturally occurring radionuclides,

nuclear waste management and the development of artificial radionuclides (IPSN, 2001; Tamponnet, 2011; Hinton, 2016). Despite the considerable body of works, many gaps exist concerning the impacts of radionuclides in the biosphere. For instance, even though the field has incorporated specialists from a range of backgrounds, their operational tools and expertise need to be expanded to enhance greater understanding of the impacts of ionizing radiation energy on soil microbiota diversity and functions.

Ionizing radiation is considered to be a unique pollutant like no other (Hinton, 2016) and several workers have adopted an ecological approach in an attempt to outline the effects of radiation on biotas and ecosystems, including Tarkhanov (1896); Gajewskaja (1923); Stoklasa and Penkava (1932); Vernadsky (1930); (1934); (1935); Crossley (1963); Odum (1963); Emelianov and Gavrilchenko (2000); Shaw (2005). The successful application of radioecological concepts in the past has led to the collection of useful data and an improved understanding of the impacts of past radiation-related accidents such as Chernobyl and Fukushima on the integrity of Earth's biodiversity and ecosystems (Møller and Mousseau, 2013; 2015). In addition, it has enabled the development of useful frameworks (ICRP, 2002; 2003; 2007; 2008; 2009) for managing actual and potential radiation exposure situations in the environment (Pentreath, 2009). The extended aim of the framework is to prevent deterministic effects and minimize stochastic effects on the environment from radiation exposure (Martinez, 2014). However, despite the use of soil as a medium for the disposal of treated and untreated radioactive waste through slow release (seeping) or deposition in large pits dug in the soil (Reichle and Auerbach, 2003); these knowledge frameworks contain little to no specific information about soil microbiota (ICRP, 2009). This is likely due to the near lack of information on the impacts of radiation on the soil systems. Yet, cases of soil contamination by radioactive pollutants are increasingly common (Prăvălie, 2014; Smičiklas and Šljivić-Ivanović, 2016).

1.1.2. Soil Contamination by Gamma Irradiation

Naturally occurring radioactive substances can be found globally, but humans are also producing more artificially. One such product and the focus of the study is ^{60}Co (Cobalt-60) gamma radionuclides, which are a man-made activation product produced from the essential trace element (cobalt) with common isotopes ^{57}Co, ^{58}Co and ^{59}Co. Although these Cobalt isotopes have found applications in many industries, little is known about how to effectively manage potential contamination incidents in personal, personnel and public terms. Gamma rays are pure energy with neither mass nor charge but are capable of travelling at the speed of light with high penetrating power enough to penetrate cells and internal organelles. High gamma radiation (~ 300,000 Gy) conditions are analogous to conditions in Mars due to the nature of the Martian atmosphere (Bank et al., 2008) and organisms that are capable of tolerating these doses may hypothetically be able to survive on Mars. Thus, radioecological studies involving gamma irradiation have implications for space exploration.

Moreover, some gamma-ray emitters have one of the longest half-lives in soil such as Potassium-40 and Argon-40 (Auerbach, 1993; Whicker and Schultz, 1982) and possibly with greater effects on soil microbiota. Considering that, the soil is important as a sink to many biogeochemical cycles, as a habitat for micro and macro-organisms, a medium for food production, and for overall ecosystem health. Soil contamination with gamma emitters like ^{60}Co will likely have major impacts on the terrestrial food chain, nutrient cycling, global biodiversity and overall biosphere functionality.

High concentrations of gamma radiation can sterilize soil but have limited influence on the structure and physicochemical properties (Horowitz and Hulin, 1971, Trevors, 1996; Berns et al., 2008). Hence, gamma irradiation is considered as a less invasive, non-phytotoxic method of soil sterilization over conventional autoclaving methods. In plants, it has been reported that low doses of gamma irradiation induce metabolic mechanisms, expressions and regulations of

genes that enhance growth and heavy metal tolerance and have been exploited in mitigating heavy metal toxicity (Hayashi et al., 2014; Wang et al. 2017). However, it remains to be seen if soil microbiota contributes to this radiation response in plants cultivated under low doses of gamma irradiation. Most small soil animals generally cannot tolerate doses greater than the background levels because reported that small to moderate irradiation doses can induce the cleavage of carbon-carbon bonds (Bank et al., 2008; Laurenco et al., 2012).

Natural soil contamination by gamma radiation has occurred across the globe with varying severity depending on the geological, geographical, biodiversity status and physicochemical conditions of the environment as well as the origin of the soil (UNSCEAR, 2000; Akhtar et al., 2005). Cobalt-60 is found in liquid effluents discharged from nuclear power and waste reprocessing plants (IRSN, 2010). In addition, the increased use of ^{60}Co gamma irradiation for the sterilization of household and laboratory consumables, wastewater treatment, sludge irradiation, preservation of historical artefacts, and nuclear power generation in Korea Republic and other parts of the world (Chmielewski, 2005; Gautam et al., 2005; Severiano et al., 2010; Varshney, 2016) is the reason why it is adopted for this study. However, this study is focused on recording the impacts of irradiation on soil microbiota and the interacting systems. Recently, Lee et al. (2017) reported the presence of increased gamma radiation in the environment of Ulsan city due to a high number of nuclear power plants. Hence, it is important to study the impact on the soil system to alleviate public anxiety regarding exposure to artificial radionuclides in the vicinity of their homes (Yoo et al., 2017). There is a global precedence of radioactive soil contamination due to radionuclide application or the discharge of their by-product and the characterization of such polluted soil systems is essential to preventative remediation and restoration (IAEA, 1998).

1.2. Application of DNA Sequencing Techniques in Soil Radioecology Studies

The advent of deoxyribonucleic acid (DNA) sequencing has fostered a greater understanding of microbial ecology and is complemented by the large microbial diversity in soil (Singer et al., 2017). However, until recently it has not been applied in the field of radioecology to capture the full range of activities and responses that may result after soil contamination by ionizing radionuclides including microbial community structure, population complexities, and functional gene activities and abundance. The suitability of DNA sequencing techniques to enumerate the extent of ionizing radiation impact is summarized by the fact that the energies involved are sufficient to break DNA strands and disrupt normal cellular activities. Thus, these techniques can advance our knowledge and understanding of hidden functions and taxonomic compositional changes in a soil microbial community after ionizing radiation pollution (Nikrad et al., 2016; Singer et al., 2017).

A previous report by Brown et al. (2015) suggests that microbial communities can determine the biogeochemical fate of radionuclides in a contaminated soil by harnessing the energy or changing their forms and physiological activities. Therefore, soil sampling and analyses through advanced sequencing technology is capable of elucidating the effects of ionizing radionuclide contamination of any study area.

There is a paucity of knowledge concerning the functional response of and interactions between soil microbes (Ellis, 2003) and this is exacerbated by a lack of understanding of the impact of radionuclides like ^{60}Co. However, the advent of novel sequencing technologies coupled with bioinformatics advancements enables the direct extraction and characterization of hidden microbial communities through DNA, PCR amplicon surveys and metagenomics has revolutionized the study of environmental microbiology and microbial ecology (Nesme et al., 2016).

1.2.1. "-Omics" Approaches and Strategies

"-Omics" is an umbrella term for diverse high throughput sequencing processes, including (meta) genomics and (meta) transcriptomics, as well as metabolic and (meta) proteomics (Walsh et al., 2017). Other subfields include epigenomics, lipidomics, interactomics, metabolomics, foodomics, diseasomics, wastomics, contaminomics, etc. Advances in "-omics" strategies and techniques have been associated with parallel breakthroughs in genome sequencing, bioinformatics, analytic tools, systems and platforms. Most notably, perhaps are developments in high-throughput technologies that have made it possible to obtain insights into microorganisms, compounds and processes in different environments (Roemer and Boone; 2013; Tang, 2015; Dos Santos et al., 2016). The study of gene-related functional responses, evolution, genome mapping through characterization and quantification of genes that coordinates the production of proteins are also within the realm of "-omics". Furthermore, "-omics" driven advances are changing the face of biological research, especially with their applications in different subfields like radioecology, improvement in sequencing methods and reduction of operational cost. Put together, these have led to ground-breaking insights about the microbial constituents of different environments including air, food, soil, and water (Frias-Lopez et al., 2008; Fierer et al., 2012; Ercolini, 2013; Bokulich et al., 2016; Walsh et al., 2017).

Since its development in the 1970's, sequencing technology has undergone major improvements, especially in speed and cost. The current sequencing technology is popularly referred to as next-generation sequencing (NGS) with the most common ones being Illumina Inc. (Genome Analyzer, HiSeq series, MiSeq, NextSeq 500) and the Ion Torrent Personal Genome Machine, GS FLX by 454 Life Sciences/Roche diagnostics, and SOLiD by ABI (Reuter et al., 2015; Ambardar et al., 2016). Different sequencing platforms are distinguished by their unique chemistry and operational principles for template preparation (source nucleic

acid extraction, library preparation and template amplification), and sequencing method (by synthesis or by hybridization and ligation). Each technique has specific pros and cons, with regards to throughput read length, error rate, coverage, cost and runtime (**Table 1**), so the choice depends on specific research aims (Loman et al. 2012; Ambardar et al., 2016; Walsh et al., 2017).

Table 1. Sequencing platforms and the average throughput lengths.

Sequencing platform	**Average read length (bp)**	**Accuracy**	**Amount of runs/reads**	**Average time**
Sanger Chain termination	~ 750 bp	99.9 %	NA	0.3-3 hours
PGM Ion Torrent	~ 400 bp	98.0 %	~80 million	2 hours
Roche 454 pyrosequencing	~ 700 bp	99.9 %	1 million	24 hours
Illumina synthesis	50 -300 bp	98.0 %	~ 3 billion	1-10 days
SOLiD ligation	50+50 bp	99.9 %	1.2 – 1.4 billion	1–2 weeks

Key: *bp = base pairs

Amplicon sequencing (single organism genome sequencing) and whole-metagenome shotgun (i.e. whole environmental community) sequencing are covered by genomics and metagenomics respectively. The sequencing depth of both approaches may be different but the general procedure is similar (**Figure 1**).

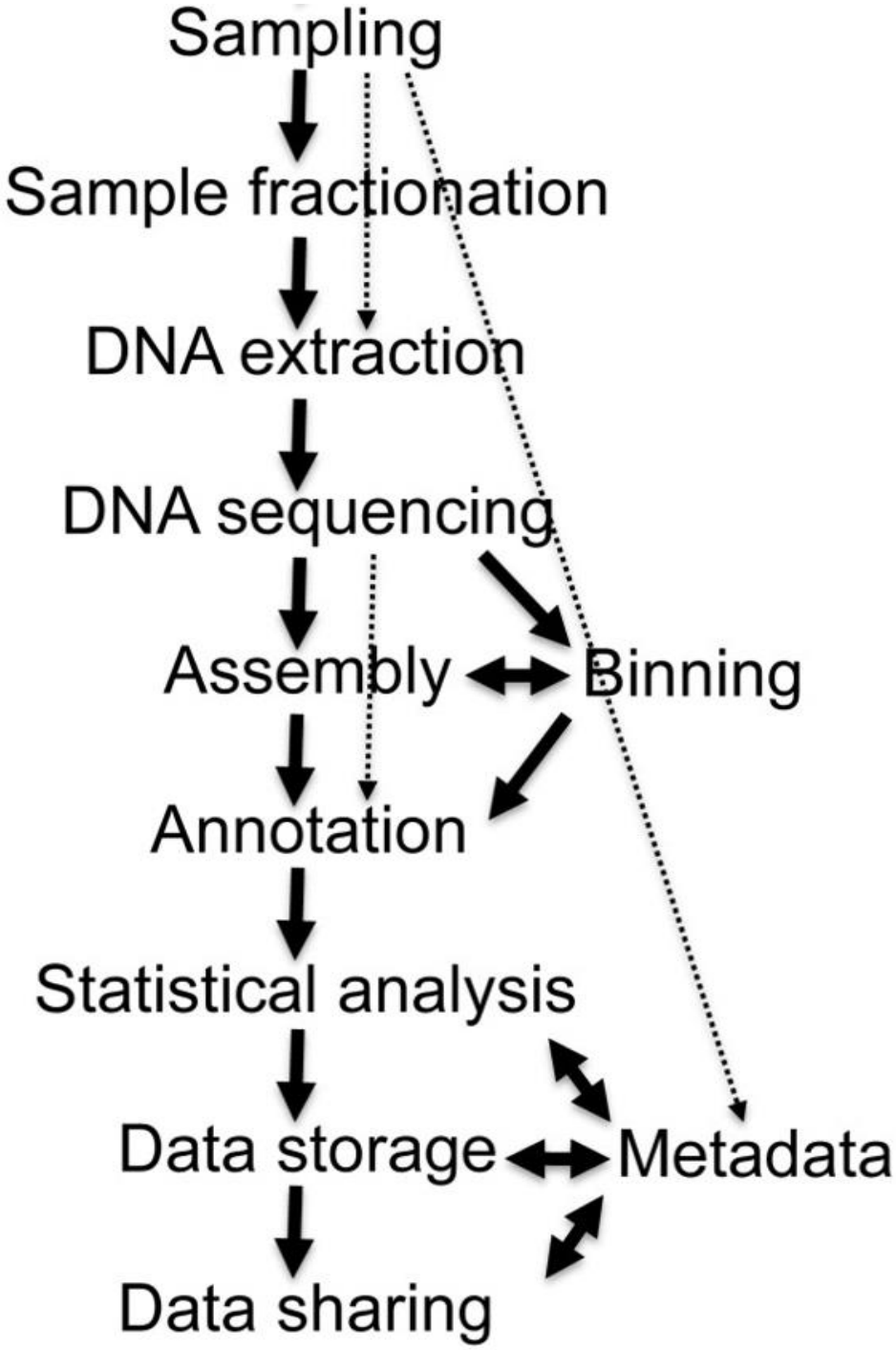


Figure 1. General outline for amplicon and metagenomics analysis of soil microbial communities.

Source: Adapted from Thomas et al. (2012)

From these processes, the taxonomic and functional (i.e. metabolic profile) constituent of a target community can be elucidated. In amplicon sequencing (e.g. 16S rRNA and internal transcribed spacer (ITS)), regions in the marker genes are PCR amplified from DNA extracted from a mixed microbial community, sequenced and aligned against a reference database to determine the taxonomic composition of a sample (Walsh et al., 2017).

Metagenomics is the application of modern genomic techniques to study communities of microorganisms directly in their natural environments, bypassing the need for isolation and the laboratory cultivation of individual species by cloning and analysing their genomes (Chen and Pachter, 2005). Pre-existing knowledge of microbial life is based on organisms raised in a pure culture and a significant percentage of microorganisms cannot be cultured in the laboratory (Sabree et al., 2009). The study of microorganisms that pervade every part of this planet has encountered many challenges through time such as the discovery of unknown organisms and the understanding of how they interact with their environment and the evolution of sequencing technology is likely to be an important contributor to solve different problems (Escobar-Zepeda et al., 2015). According to Handelsman et al. (1998); Thomas et al. (2012) metagenomics is among the fastest growing scientific disciplines and has become a central tool for improving life worldwide through the fields of microbial ecology, evolution, and diversity. The reason for promoting the use of metagenomics to understand the taxonomic composition and functional interaction of radionuclide-contaminated soil is that only a small proportion of organisms have been grown in culture and many species do not live in isolation; they form complex interacting communities. The failure of clonal cultures can be addressed through the capability of metagenomics to record hitherto undiscovered diverse microbial communities of bacteria, archaea, viruses and single-celled eukaryotes that are crucial to the soil system.

1.2.2. Soil Microbiome Legacy Effects in a Contaminated Environment

Microbiomics offers a unique opportunity to understand and improve our knowledge of the hidden world around us by elucidating the interactions that it composes (Biteen et al., 2016). Soil microbiomes are essential to human and ecosystem health (Ochoa-Hueso, 2017). Qualitative and quantitative alterations in the metabolic activities and distribution of soil microbiota can result from exposure to various environmental factors and are described as edaphic dysbiosis (Hawrelak and Myers, 2004; Carding et al., 2015; Minich et al., 2018). While the concept of dysbiosis has been widely studied to indicate health status (García-Castillo et al. 2016; Levy et al., 2017; Hooks and O'Malley, 2017), there is little to no report on how the initial composition and activity of soil system change after a radiation-induced contamination. These studies will report on edaphic dysbiosis and the resulting legacy outcome caused by an ionizing agent (^{60}Co gamma radiation) using an "-omics" approach.

The 'legacy effect' describes the persistence of functional and compositional alterations in the (soil) ecosystems following a disturbance event and has been documented for over a century (Gauvreau, 2017). Edaphic dysbiosis may not be the direct outcome of the disturbance, rather the outcome of important interactions between the microbiome as a result of external stressors such as irradiation (Carding et al., 2015). However, the effects of these disturbance-induced altered interactions persist by leaving a legacy effect in the community through dead cells and biochemicals that influence the physicochemical processes of the surviving (successional) population. Thus, below and above ground externalities can affect soil microbial community composition and diversity. Hence, distinct soil communities can result from both external and internal stressors. The legacy or residual effects of the stress may be short or long-lived depending on the resulting community (i.e. legacy response assemblage) and the nature of the stress. To measure 'legacy effects', it is paramount to ascertain the duration and nature of the legacy causing factors and what they influence (Zhang et al., 2018). In these studies,

ionizing gamma irradiation is anticipated to impose a lasting signature on the soil microbial community, but the extent and amplitude of their contribution to the soil microbiome remains unclear because of the differential response, resilience, tolerance level and recovery patterns of soil microbial assemblage (Gauvreau 2017; Jurburg et al., 2017; Meisner et al., 2018). Moreover, opportunistic bacteria like radiation-resistant, Deinococcus-Thermus may exploit the novel conditions to develop a unique climax community and colonize the soil. However, this is complicated by the rapid rate of microbial turnover and the historical range of microbial ecosystems versus the duration and depth range of stress intensity (Banerjee et al., 2016). Nonetheless, these studies will assess the contributions of abiotic and biotic processes to the overall legacy outcomes through the surviving soil microbial communities (Wong et al., 2018). Knowledge about the surviving groups will advance knowledge about the soil quality post-disturbance.

1.3. Research Aims and Objectives

The ecological fate of ionizing radionuclides that enter the terrestrial environment mainly includes soil deposition and soil-to-plant transfer (Zhu and Shaw, 2000). Yet, little is known about how the soil system reacts to overcome the stress caused by this deposition. This research is set up with the prime objective of understanding how soil chemical and microbiota react to repeated exposure to different gamma radiation intensity.

This research will adopt a novel community based "-omics" approach for assessing the effects of gamma irradiation on the soil ecosystem and produce supporting data regarding the fate of the soil microbiota. Functional genes and taxonomic changes between and within the different soil microbial communities of the treatment groups will be used to assess the nature and level of the impact caused by the radionuclides. Whereas an undisturbed soil system may develop climax communities easily, a radionuclide-contaminated soil is expected to produce a climax community that reflects the level of disturbance. Therefore, this study will reveal how the soil productivity may change after irradiation incidence. In addition, the study will consider changes in some essential soil chemicals. This knowledge can be used to implement soil rescue missions and management in case of soil contamination by radionuclides.

In the first part of the study, changes in microbial functional genes and the taxonomic composition of an early successional soil exposed to gamma radiation will be reported from a metagenomics standpoint (Part 2). There has been very limited prior research concerning the taxonomic and functional response of soil microorganisms (at the community level) to ionizing radiation. Though the majority of soil microorganisms are unculturable and as yet unidentified, they carry out important functional processes that can be used to infer their presence (Jansson and Hofmockel, 2018). To address this, the second part of the study will adopt a metagenetics approach in an attempt to understand how bacterial community composition, abundance and

diversity is influenced by the dominance of radiation-tolerant taxa, Deinococcus-Thermus (Part 3).

Ge et al. (2013) have suggested that the adopted approach is able to measure chemical toxicity and soil microbiota response with practical application in environmental risk assessment and monitoring. Hence, as part of its objective, these studies sought to enable a reliable prediction of soil compositional status following gamma irradiation. This study will contribute to the understanding of soil microbial response to ^{60}Co gamma irradiation and will aid in the prediction of taxonomic and functional gene changes in a soil contaminated by gamma irradiation. This, in turn, enables us to understand changes in the natural environment following the accidental and purposeful release or leakage of ^{60}Co containing materials.

Furthermore, the study will increase public awareness about the magnitude of changes that may result from gamma irradiation of soil and improve on available data on soil irradiation effects that can be adopted by project developers, researchers and policy formulators in the management of nuclear accidents. These data can also be utilized to predict the effects of radionuclide contaminants and protect the soil from actual and potential contamination activities. In addition, the methods utilised by these studies reported here may serve as a model for assessing natural accidents involving radionuclides and other emerging pollutants.

PART 2. CHANGES IN SOIL TAXONOMIC AND FUNCTIONAL DIVERSITY RESULTING FROM GAMMA IRRADIATION

2.1. Introduction

The effects of ionizing radiation on humans and the ecosystems have been investigated since the 1950s (Schwartzbaum et al., 1994; Harrison and Anderson, 1994; Jones et al., 2001; Robison et al., 2003; Richards et al., 2008; Martinez et al., 2010; Kerr et al., 2013) driven by interest in understanding radiation effects on both natural and agricultural ecosystems. Radiation effects are also of interest for exploring the viability of ecosystems in space travel and space colonization (Horneck et al., 2002, 2010), and in assessing the likelihood of independently evolving biota in high radiation environments on other planets (Beblo et al., 2009, 2011).

Radiation is known to affect living cells in diverse ways. There are direct effects on the structural integrity of cellular components, such as membranes (Hollosy, 2002; Kovac and Keresztes, 2002; Kujawa et al., 2004), and on enzyme viability by protein denaturation (Cox, 1989; Cabiscol et al., 2000). Cells are generally most vulnerable when actively dividing, due to the breakage or alteration of DNA that results from radiation (Rothkamm et al., 2003). Certain types of organisms are far more susceptible than others to radiation (Cox and Battista, 2005); although some types of bacteria and archaea can survive at thousands of times the dosages that would kill humans (Koonin and Wolf, 2008). The biological mechanism of radiation resistance differs between taxa and involves the coordination of a myriad of biochemical and genetic processes. In the most radiation resistant groups known (*Deinococcus* sp. and *Thermococcus gammatolerans*), resistance involves genes for a complex network of DNA repair and metabolic switching processes (Narumi, 2003; Jolivet et al., 2003). Various radiation resistance and repair mechanisms have also been identified in living organisms (Rothschild and Mancinelli, 2001).

However, the effects of high doses of ionizing radiation on complex communities of soil organisms have not been well studied. Soil is arguably the most biologically diverse

environment on Earth (Daniel, 2005), and most of its diversity consists of complex communities of organisms that are microscopic or near-microscopic, and morphologically cryptic (Castaneda and Barbosa, 2017). Most of these microscopic life forms have neither been isolated nor studied (Sabree et al., 2009; Pershina et al., 2016), yet these microorganisms together play key roles in the ecosystem (e.g. Lisitskaya and Trosheva (2013); Gourmelon et al. (2016)). A more thorough assessment of soil biological diversity, including the huge numbers of non-cultured forms, is now possible due to advances in sequencing and bioinformatics. Using environmental DNA-based methods to study irradiated soils has the potential to uncover the full range of radiation-resilient forms of every group of organisms (including bacteria, fungi, metazoa, protists, archaea or viruses), and the basis of radiation resistance amongst these diverse groups of organisms.

The study of irradiated soil communities also may be approached from a generalized ecological point of view, as a system subject to stress and disturbance effects followed by recovery. This approach has the potential to provide clues to the processes that govern the assembly of communities in nature, including the mechanisms behind diversity and coexistence. This study follows this approach, focussing on a number of hypotheses regarding the effects of radiation on soil communities and their functional characteristics:

1. Soils subjected to gamma irradiation will have a taxonomically distinct biota from control soils, and the diversity of this biota will differ according to the radiation dose received.

It is expected that exposure to radiation will lead to lower taxonomic diversity as high radiation is a universal stressor to which microbial communities have low overall adaptation and tolerance (Salbu and Skipperud, 2007). Survival at high doses of radiation appears to involve elaborate mechanisms that probably evolved in response to natural exposure to UV radiation, drying and extreme heat (Billi et al., 2000; Rothschild and Mancinelli, 2001;

Makarova et al., 2001). It is likely that only a subset of taxa may happen to carry the adaptive traits to survive a large dose of radiation, which will result in a progressive diminution of diversity. Furthermore, it is hypothesized a large shift in the taxonomic composition of the soil biota, in terms of both lower and higher-level taxa, as some taxa will happen to possess stronger adaptations for radiation tolerance than others do. It is hypothesized that at the highest level, Bacteria and Archaea would become relatively more abundant than Eukaryota, and especially Metazoa, due to the simpler cellular organization of these organisms (Errico and Costanzo, 2010).

2. Soils exposed to radiation will have a lower diversity of functional genes owing to the reduced diversity of taxa that can survive, and the restrictions imposed by radiation damage to physiological/ecological strategies.

It is anticipated that the extreme physiological and biochemical challenges presented by repeated radiation exposure would result in a reduced range of functional genes comprising the metagenome. Partly, this would be an incidental effect of reduced taxonomic diversity – reducing the range of genes particular to individual taxa. However, it is also hypothesized that the damage caused by high radiation would make a range of energy-requiring physiological or biochemical functions non-viable (Howland, 1998), because so much of the energy and resources of the cell would be diverted into radiation damage protection and repair mechanisms, thereby restricting the potential range of niches and ecological strategies.

3. A greater abundance of certain key groups of genes will be associated with radiation exposure.

These are likely to include stress-response genes, which assist in the survival of various extreme conditions such as high temperatures, freezing or high salinity through forming

biofilms, fruiting bodies, filaments, spores or taxis responses – often through regulating other downstream genes (de Bruijn, 2016; Wexhselbaum et al., 1994). It is envisaged that this occurs mainly through pre-adaptation after having evolved in the organisms to cope with damage caused by other stressors, and organisms carrying such genes are then ecologically selected, becoming more abundant in soil exposed to radiation.

Dormancy and sporulation-related genes were also hypothesized to become more abundant because actively dividing cells are known to be particularly susceptible to radiation damage (Little, 2003). However, resistant species have the capacity to outlive it while others with the capacity to form resting spores or to enter periodic dormancy will be adapted to avoiding radiation damage (Battista, 1997; Zhang et al., 2015). Dormancy allows organisms to recover following disturbance in a way that other taxa cannot. Since radiation exposure in the experimental systems occurred in repeated bursts, rather than continuously, cells that were coincidentally dormant during exposure phases would be more likely to survive and then become abundant.

It is also anticipated that there will be a lower abundance of competition-related genes associated with radiation exposure. Each 24-hour radiation dose is likely to be associated with phases of mass death of cells, followed by a recovery phase in which surviving forms recolonize, exploiting the nutrients available from dead cells. This would be an example of an 'r' selected environment (Pianka, 1970; Andrews and Harris, 1986), and such environments with abundant nutrients and space, are seen as ecologically selecting for rapid growth and reproduction, rather than interference competition. Consequently, it is expected to see a lower abundance of genes relating to antibiotic production (or antibiotic resistance to cope with this), and lower abundance of genes relating to cell-cell interactions (characterised as regulation and cell signalling, which includes programmed cell death and toxin-antitoxin systems related

genes, proteolytic pathways related genes, quorum sensing and biofilm formation and regulation of virulence).

Additionally, as part of this study, basic soil parameters – total organic carbon, total nitrogen, pH and total phosphorus – were investigated to understand the extent to which the chemistry of the whole soil system may change either because of gamma (γ) ray exposure to the organisms (Blankinship et al., 2014), or due to the effects of the radiation on soil chemistry. Chemical changes in soils that are completely sterilized by γ- rays are generally considered to be relatively minor (Eno and Popeno, 1962, 1964; McLaren, 1969; Wolf and Skipper, 1994; Tuominen et al 1994; Alef and Nannipieri, 1995; Thompson, 1990; McNamara et al., 2003, 2007). However, γ-irradiation has been shown to induce surviving microbes to decompose soil matter, by influencing their cellular metabolism and functionality without altering the soil structure (Schimel et al., 2007; Blankinship et al., 2014). This aspect of the study will also investigate the emergent properties of a changing soil system characterized by the survival of some living forms, resulting from repeated bursts of exposure. Whether these changes are solely due to purely chemical effects of repeated exposure to γ-rays, or consequences of shifts in soil biota community and activity might not be clear. Nevertheless, the possibility of such changes should be investigated as a part of the entire system, even if only as a spur to further study.

2.2. Materials and Methods

2.2.1. Study Sites and Soil sampling

The soil was derived from an early successional environment on the Seoul National University campus (37°27′38.3″N, 126°57′07.5″E), located in the Gwanak Mountain area, South of Seoul, Republic of Korea. The site is characterized by a cool humid temperate climate (MAT 13.3 ^{o}C, MAP 1,212.3 mm). The vegetation of the sampling site is composed of around 80% ground cover by common ruderals such as *Poa, Festuca, Artemisia, Taraxacum, Senecio* and *Capsella*.

Sampling was carried out in the early winter season (December 2014), when soils were not yet thawed. The soil is a sandy loam typical of that region of Seoul. Three quadrats (each 10 × 10 m in size) were established 50 m apart along a linear transect. Approximately 100 g of soil (from 0 to 10 cm depth) was collected from the four corners and one centre point of the quadrat and pooled to make one individual sample. Overall, 15 samples were collected from within the three quadrats (five samples from each). Thereafter, the collected soil samples were homogenized and sieved with a sieve of 2-mm mesh size and stored at ambient room temperatures for approximately 24 h until they were incubated or irradiated. Using a completely randomized experimental design with five treatments. These were: pre-treatment (initial pre-experiment) soil from before treatments, Control (no radiation exposure but held in the incubator for six weeks) and the Low, Medium, and High radiation after six weeks. There were three replicates for each treatment (except for pre-treatment, which had 12 replicates). For each replicate, 500 g soil was placed in a ceramic self-draining pot.

2.2.2. Soil Incubation and Gamma [^{60}Co] Radiation Treatment

Immediately after sample collection, 100 g of the initial pre-treatment soil sample was used for chemical analysis and DNA extraction. Replicates of this pre-treatment soil were also

stored untreated for 6 weeks alongside the pots containing the irradiated soils, during the course of the experiment. The soil was stirred once a week in all pots, whether radiation-exposed or not. The free-draining pots were then all stored in an incubator at 25 ^{o}C, in randomized positions. Each pot was watered with 200 ml of deionized water every three days – which was enough to keep the pots close to field capacity in moisture.

The samples for radiation-treatment were each exposed to one of three different levels of gamma ^{60}Co radiation treatment at Korea Atomic Energy Research Institute [KAERI] (https://www.kaeri.re.kr/english/sub/sub03_04_01_06.jsp). The intensities of gamma ^{60}Co radiation treatment applied to the soil were low (at 0.1 kGy), medium (at (1 kGy) and High (at 3 kGy). During irradiation, they were held in 500 ml clay pots. The radiation treatment was carried out for 24 hours without a break, once a week for six weeks with a cobalt-60 γ-ray irradiator (point source, AECL, IR-79, Canada). The distance between the samples and radiation source was adjusted based on the γ-ray intensity and dose to give a uniform treatment/effect. The soils were then mixed well to ensure homogeneity before the next treatment. Cross contamination was prevented by keeping the pots apart (and not above each other) on the same level, using different sterile rods to stir each pot. Watering was done for each pot separately to prevent splashing. As the soils did not dry out, transmission of dust or dried spores between them was unlikely. The pots were not covered, to allow free gas exchange to the soil microbial community. The positions of replicate pots of different treatments in the incubator were randomized and randomly interchanged each week. The total amount of gamma ^{60}Co radiation received by the samples throughout the six-week period was 0.6 kGy (low), 6 kGy (medium) and 18 kGy (high) respectively.

Samples were collected from each pot (of Controls and, Low, Medium and High) after six weeks to be used for soil chemical analysis and total DNA extraction.

2.2.3. Soil Chemical Analysis

On the final day of the study, samples from each experimental and control replicate were analysed for soil chemistry variables including total organic carbon (TOC), pH, total nitrogen (TN), and total phosphorus (TP) at the National Instrumentation Center for Environmental Management (NICEM, South Korea) based on the standard protocol of the Soil Science Society of America. TOC content was determined by oxidation with 1 N potassium dichromate in an acidic medium, according to Rowell and Florence (1993); Rowell (2000; 2014). pH was determined using a combined pH electrode in a soil-water suspension (soil/tap water = 1:2). TN was determined by sulfuric acid digestion using Se, $CuSO_4$, and K_2SO_4 as catalysts, with 1 g of soil with final TN content in the digest determined by the regular Kjeldahl distillation method (Thomas, 1982). Available phosphorus was determined by the method of Bray and Kurtz (1945) by autoanalyzer with 3 g of soil.

2.2.4. Total DNA Extraction and Shotgun Metagenomic Sequencing

The soil DNA was extracted from 0.50 g sample of soil from each sample in replicates, using the Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) following the protocol described by the manufacturer. DNA isolated from each sample was amplified using primers 338F (5'-XXXXXXXXGTACTCCTACGGGAGGCAGCAG-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3'), targeting the V3 hypervariable regions (Huse et al., 2008). The Polymerase chain reactions (PCR) were carried out under the following thermal profile: denaturation at 94 °C for 2 min, followed by 25 cycles of amplification at 94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. PCR products were analysed by electrophoresis in 1 % agarose gels and were purified using Wizard SV Gel and PCR Clean-up System (Promega, USA). The paired-end sequencing for the whole metagenome was carried out using Illumina HiSeq 2000 sequencing system platform (2 × 150 bp) (Illumina) according to the manufacturer's instructions at Celemics (Celemics, Seoul,

Korea). Library preparation, sequencing, and initial quality filtering were performed as described previously in Zhou et al. (2011).

2.2.5. Data Processing

To annotate the unassembled DNA sequences, the Metagenomics Rapid Annotation using Subsystems Technology (MG-RAST) pipeline (Meyer et al., 2008) was used. The MG-RAST pipeline includes several quality control filtering options for DNA sequence data, including removal of artificial duplicates, reads, and quality-based and length-based read trimming. The M5 non-redundant protein database (M5NR) was used for taxonomic annotation and the SEED database and Clusters of Orthologous Groups database for functional annotation. To identify the sequences, the best BLASTx hit was used with a minimum alignment length of 15 bp and an E-value cut-off of $e < 1 \times 10{-}5$ and 95 % confidence interval. Functional annotation of the most abundant taxa was performed using the filter option. The same was done for a select group of genes to reveal the responsible taxa. The shotgun metagenomics sequence data used in this study are deposited in the MG-RAST server under project ID 20322 (http://metagenomics.anl.gov/linkin.cgi?project=mgp20322).

2.2.6. Quantitative Reverse Transcription Polymerase Chain Reaction

To investigate the effects of radiation on the absolute abundance of the soil biota, I conducted quantitative real-time PCR (qPCR) amplification of gene copy numbers for two important groups of soil organisms. The qPCR was done on unamplified DNA samples using Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR system (by Life Technologies, Carlsbad, CA) to measure the proportion of (1) all bacteria, and (2) all fungi. To quantify bacterial 16S rRNA gene the forward primer 5′-TCCTACGGGAGGCAGCAGT-3′ and the reverse primer 5′-GGACTACCAGGGTATCTAATCCTGTT-3′ were used as suggested by Nadkarni et al. (2002). For fungal quantification, the primers ITS1F (5′-

CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) were used (Gardes and Bruns, 1993; White et al., 1990). The reaction mix consisted of 10 µL of 1x QuantiTect SYBR Green master mix (QIAGEN) with HotStar Taq, 0.5 µl of each primer (10 µM), 10 ng DNA template or prepared standard and PCR grade water to a final volume of 20 µL. Thermal cycling conditions were as follows: 50 °C for 2 min, activation step at 95 °C for 15 min, followed by 40 cycles of denaturation (95 °C for 15 s), annealing (60 °C for 1 min), and elongation (72 °C for 15 s). All samples including the non-template control and dilution series of standards were run in triplicate. Results were analysed using the ABI Prism 7900HT sequence detection system (Version 2.4). Thereafter, the total gene copy numbers of 16S rRNA and fungal ITS gene copies were calculated for each sample. A 10-fold dilution series were used and PCR amplification efficiency (E) was calculated based on the standard curve using the formula: $E=(10^{-1/slope}-1) \times 100$ %. No PCR inhibition was found after checking with the methods applied in Hospodsky et al. (2010).

2.2.7. Statistical Analysis

The taxonomic and functional diversity (Shannon index), richness and evenness were calculated using 'Vegan' and 'BiodiversityR' packages in R studio (R Development Core Team, 2008; Oksanen et al., 2014). To assess the interactions in the samples, ANOVA and Tukey HSD posthoc or Kruskal-Wallis and Pairwise-Wilcox posthoc test depending on whether they were normal. Relative abundances were calculated and then used to construct a boxplot or subjected to the 'pheatmap' command in R studio. Non-metric dimensional scaling (NMDS) and principal component analysis (PCA) was performed using PAST [PAlaeontological Statistic] package (Hammer et al., 2001) to investigate the relationship of ionizing radiation exposure to the soil chemistry and community composition of each treatment.

2.3. Results

2.3.1. Soil Chemical Properties

Soil chemical properties differed among the treatment levels with the exception of pH, which showed little variation across the treatments (**Table A1**). Pre-treatment samples had the highest TP concentration whereas, at the end of six weeks, the Control samples had the lowest with average values of 512.54 and 448.18 mg/Kg respectively. The concentration of TN was highest in the pre-treatment samples and lowest in high radiation treated samples whereas TOC concentration, which was also highest in the pre-treatment, was lowest in the low radiation treated samples.

TOC and TP were significantly different between the treatment groups with $P\text{-}_{values}$ (at $p \leq 0.05$) (**Figure A1**). To understand the overall correlation effects, a principal component analysis was conducted (**Figure 2**). This factorial analysis suggests that pH and TOC accounted for 79 % of the observed variations in the chemical parameters.

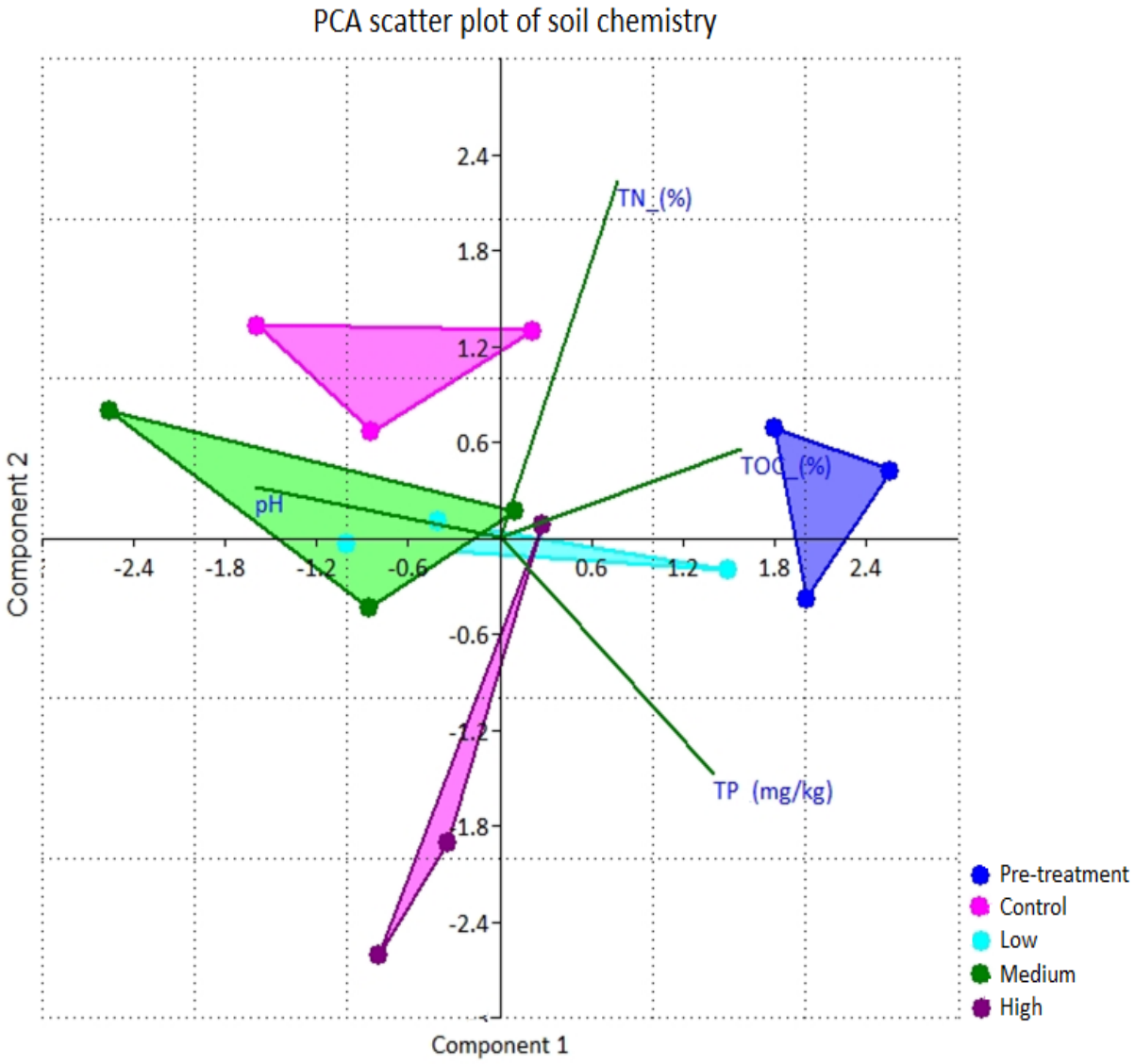


Figure 2. Principal component analysis of the effects of gamma irradiation on the measured environmental variables. Component 1 and 2 account for 79.802 % of the observed variation. PCA loadings of component 1 suggest TN, TOC and TP loaded positively the axis and are responsible for its influence while TN has the most effect on component 2.

2.3.2. Microbial Taxa Abundance and Community Composition

Approximately 65 million good quality sequences were obtained after quality control filtering from 24 samples through shotgun metagenomic sequencing (**Table A2**). Of these, 40 – 45 % of the total metagenomic sequences were annotated to a protein of known function using E-value $<1 \times 10^{-5}$ and 15-bp minimum alignment length.

Relative abundance of domain and dominant phyla shows large differences between bacteria, archaea, and eukaryotes. Bacterial sequences were predominant in every sample (98.36 % of all sequences, overall) followed by Archaea (1.21 %) and Eukarya (0.43%) (**Figure A2a**). The relative abundance of phyla suggests different patterns of abundance within and between treatments (**Figure A2b**). Functional annotation of major domains reveal different sets of dominant coding genes in each domain (**Figures A2c** and **d**). The dominant ones include clustering-based subsystems, carbohydrates and amino acids and their derivatives-related genes.

Among the 21 recognized bacterial phyla recorded in the study, Deinococcus-Thermus was the most abundant (5.59 % on average), followed by Chloroflexi (4.33 %), Actinobacteria (4.09 %) and to a lesser degree Chlamydiae (3.91 %) and Cyanobacteria (3.90 %) (**Figures 3** and **A3a**). Proteobacteria was the most abundant bacterial phylum in the pre-treatment and control samples whereas Deinococcus-Thermus was the most abundant in the highest irradiation treatment. Functional annotation of these abundant phyla reveals that although the diversity (in terms of number) of coding gene types was similar, they utilized higher proportions of certain types of these genes (**Figure A3b**).

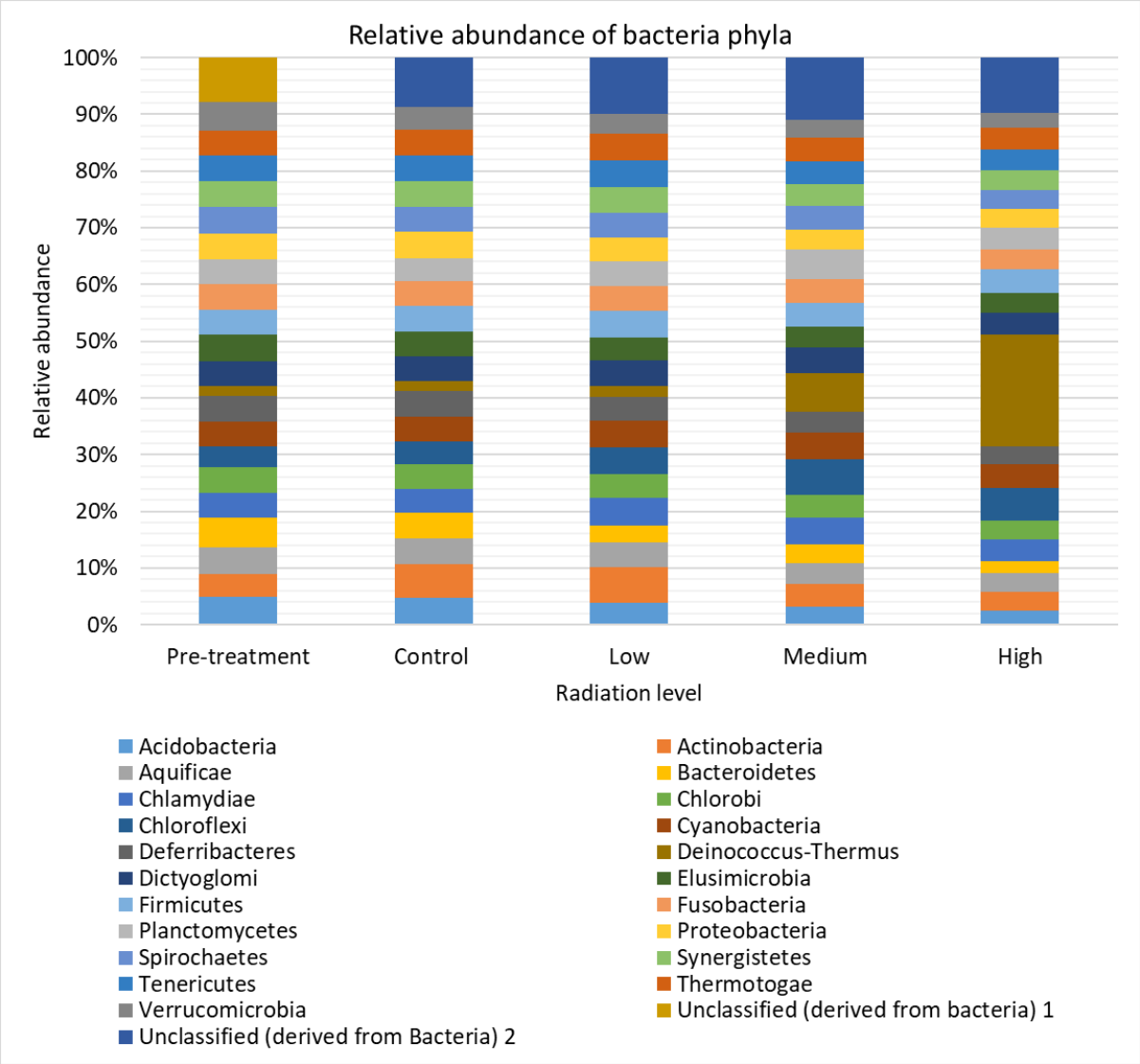
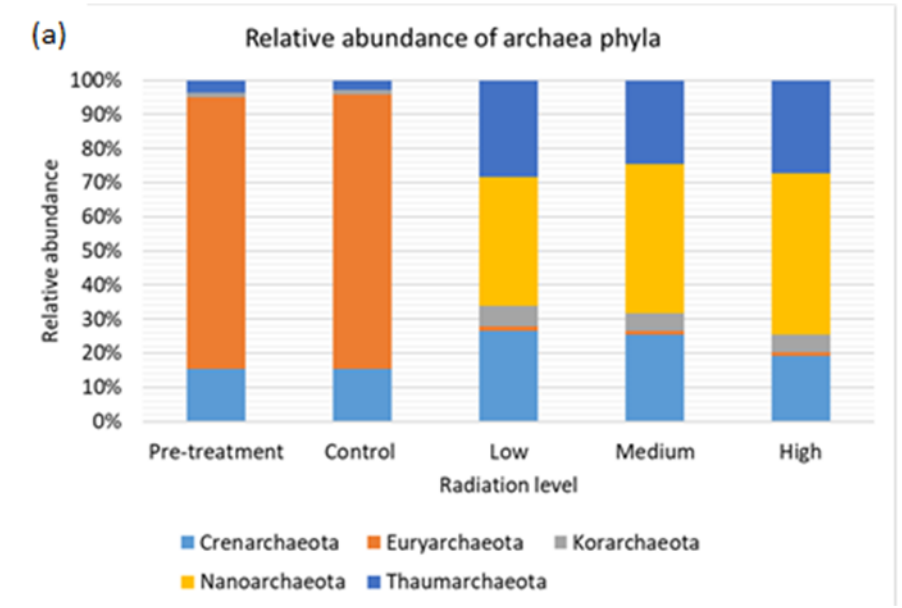
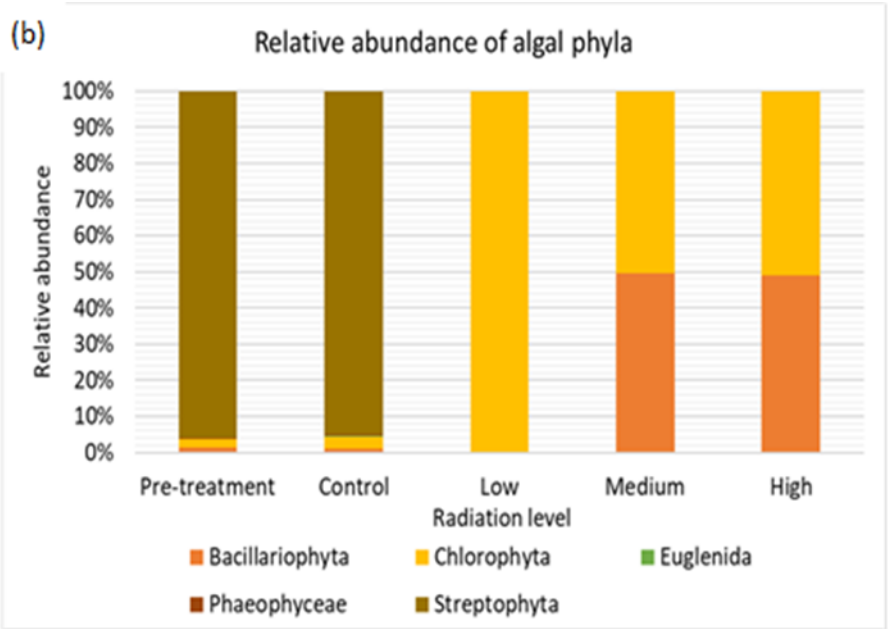


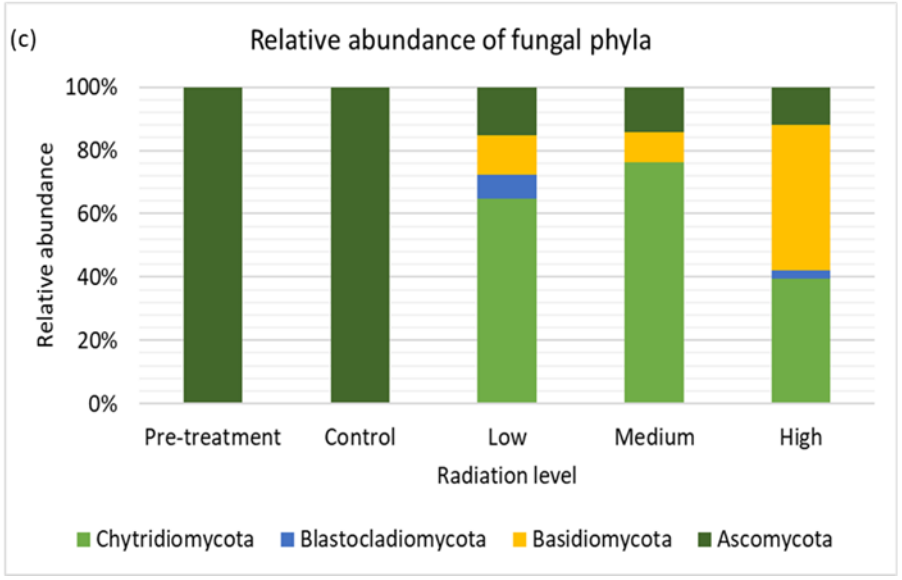
Figure 3. Relative abundance of bacteria phyla observed in shotgun metagenomics sequence data after exposure to different levels of gamma radiation.

The most abundant archaeal phylum across all treatments was Nanoarchaeota followed by Thaumarchaeota and Crenarchaeota (**Figure 4b**). In the control and pre-treatments samples, Euryarchaeota was the most abundant archaeal phylum. This phylum was severely impacted by the higher doses of irradiation as shown by its greatly reduced abundance in the radiation-treated samples compared to the other phyla (Crenarchaeota, Korarchaeota, Nanoarchaeota and Thaumarchaeota), which all increased their relative abundance. Functional annotation for Nanoarchaeota suggests a high proportion of genes related to DNA processing and protein metabolism (**Figure A5a**).

Twenty recognizable Eukaryota phyla were obtained including algal, fungal and metazoan phyla (**Figure 4a**, **c** and **d**), which all varied across the treatments. Streptophyta was the most abundant algal phylum in the control samples whereas Chlorophyta and Bacillariophyta were abundant in the radiation-treated samples (**Figure 4a**). Functional annotation of Chlorophyta reveals suggest they were actively utilizing respiration-related genes (**Figure A4a**). Among the fungal phyla, Ascomycota and Chytridiomycota were most abundant in the control and radiation treated samples respectively (**Figures 4c**). Functional annotation of Chytridiomycota suggests high utilization of respiration-related genes (**Figure A4b**). Chordata was the most abundant metazoa phylum in the control, while Apicomplexa and Echinodermata (presumably mis-assigned and from other metazoan phyla, due to the limitation in metazoan gene characterisation in existing databases) were abundant in the radiation-treated samples (**Figure 4d**).


(a)
Relative abundance of archaea phyla
Relative abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Pre-treatment
Control
Low
Medium
High
Radiation level
Crenarchaeota
Euryarchaeota
Korarchaeota
Nanoarchaeota
Thaumarchaeota


(b)
Relative abundance of algal phyla
Relative abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Pre-treatment
Control
Low
Medium
High
Radiation level
Bacillariophyta
Chlorophyta
Euglenida
Phaeophyceae
Streptophyta


(c)
Relative abundance of fungal phyla
Relative abundance
100%
80%
60%
40%
20%
0%
Pre-treatment
Control
Low
Medium
High
Radiation level
Chytridiomycota
Blastocladiomycota
Basidiomycota
Ascomycota

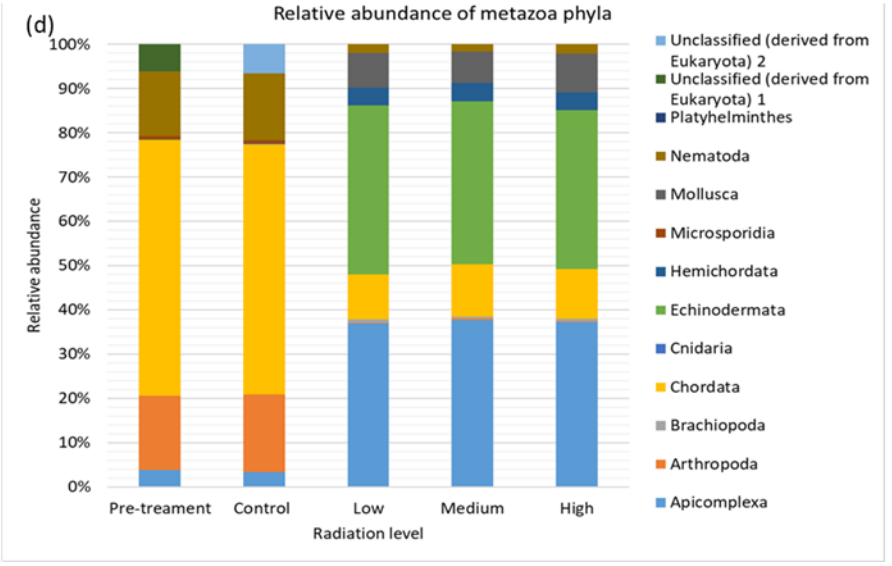

(d)
Relative abundance of metazoa phyla
Relative abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Pre-treament
Control
Low
Medium
High
Radiation level
Unclassified (derived from Eukaryota) 2
Unclassified (derived from Eukaryota) 1
Platyhelminthes
Nematoda
Mollusca
Microsporidia
Hemichordata
Echinodermata
Cnidaria
Chordata
Brachiopoda
Arthropoda
Apicomplexa

Figure 4. Relative abundance of archaea, algal, fungal and metazoa phyla observed in shotgun metagenomic sequence data after exposure to different levels of gamma radiation. A pronounced difference is observed in the abundance of all the phyla between the treated and control samples (**Figure 4a - d**). The most abundant archaea phyla in the pre-treatment and control samples are Euryarchaeota and Nanoarchaeota in the samples exposed to ionizing radiation (**b**). Streptophyta was the most abundant algal phylum in the control samples whereas Chlorophyta and Bacillariophyta were abundant in the radiation-treated samples (**a**). Among the fungal phyla, Ascomycota and Chytridiomycota were most abundant in the control and radiation treated samples respectively (**c**). Chordata was the most abundant metazoa phylum in the control while Apicomplexa and Echinodermata were abundant in the radiation-treated samples (**d**).

The data from qPCR for both bacteria and fungi reveals a decline in gene copy numbers, suggesting a substantial decrease in populations, with increasing radiation dose (**Figure 5**).

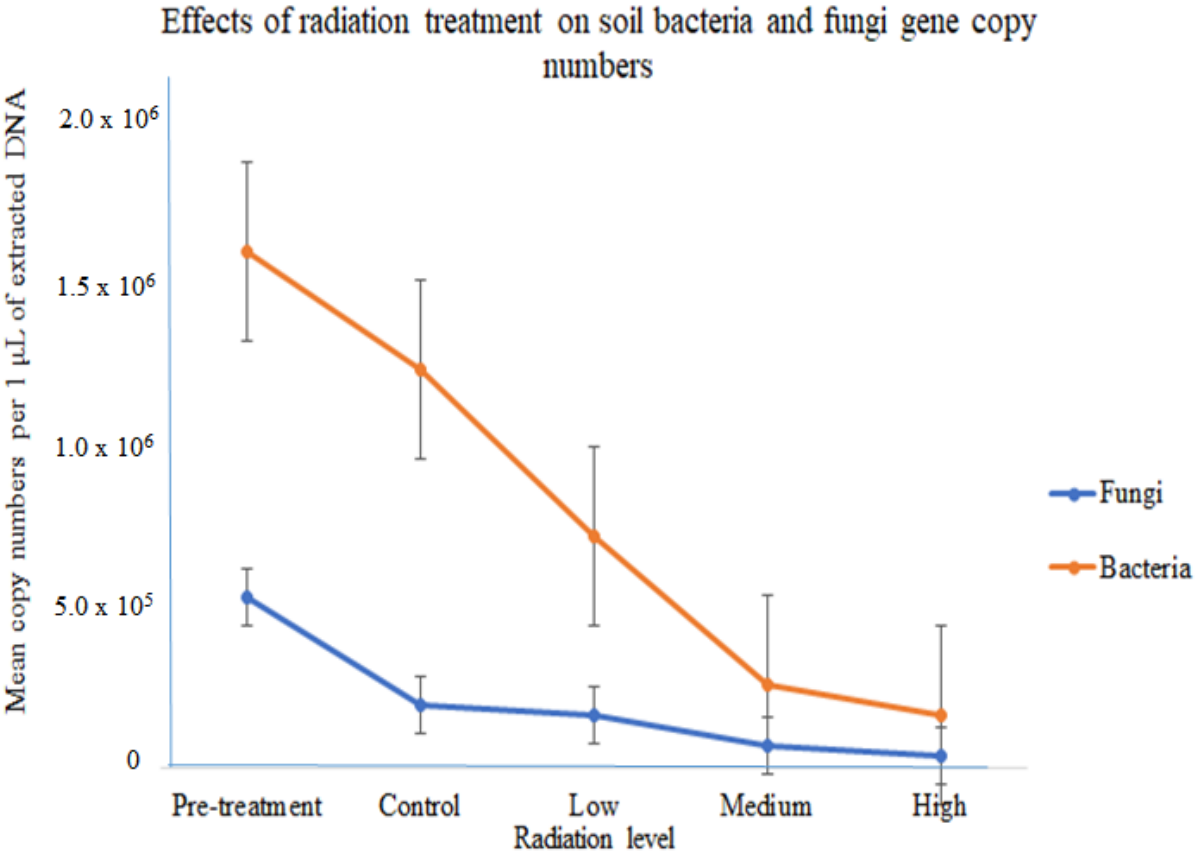


Figure 5. Effects of radiation treatment on soil bacteria and fungi gene copy numbers. The bacterial gene copy number was higher than those of the fungal however both showed similar decreasing patterns with increasing irradiation dose. Pre-treatment sample had the highest copy numbers whereas the radiation treated samples decreased with increasing radiation dose.

2.3.3. Functional Gene Abundance and Composition

Twenty-eight functional genes were obtained from SEED level 1 with varying abundance including amino acid derivatives, carbohydrates, cell division and cell cycle, DNA metabolism, dormancy and sporulation, protein metabolism, RNA metabolism, secondary metabolism, virulence and stress response-related genes (**Figure 6** and **Table 2**).

The most abundant gene category across all treatments were carbohydrates (13.59 % on average), followed by clustering based subsystems (12.83 %), amino acids and derivatives (10.41 %), protein metabolism (7.94 %), miscellaneous (6.51 %), cofactors, vitamins, prosthetic groups and pigments (5.56 %) and DNA metabolism (4.34 %) related genes (**Figure 6**). Among the 28 functional gene categories, 25 differed significantly across the treatments (**Table 2**). The gene categories associated with core metabolic functions under adverse conditions such as cell walls and capsules, clustering-based subsystems, DNA metabolism, dormancy and sporulation, virulence and stress response were significantly affected (at $P \leq 0.05$) by the treatments (**Figure 6** and **Table 1**).

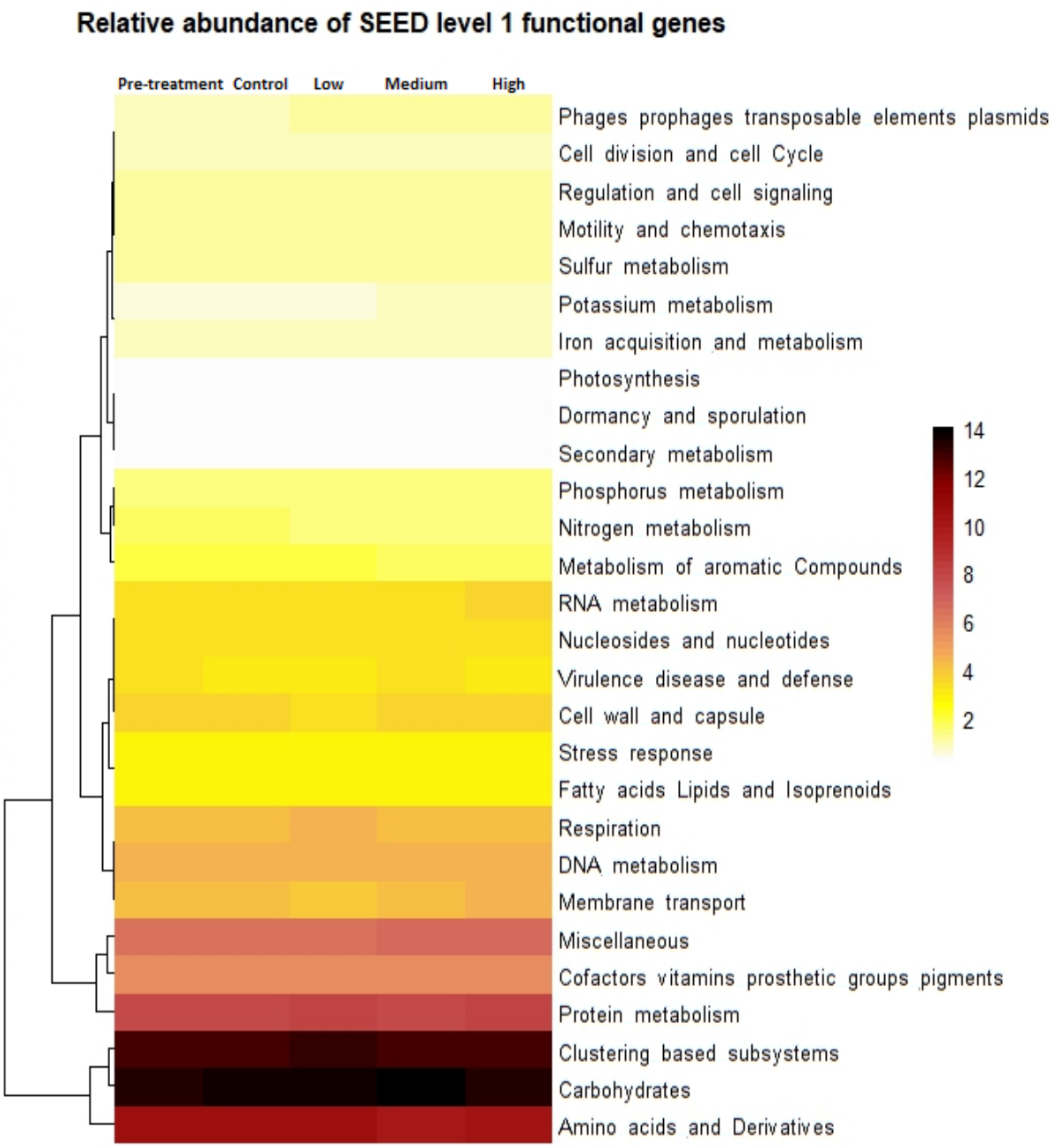


Figure 6. Relative abundance of SEED subsystem level 1 functional genes observed in shotgun metagenomic sequence data after exposure to different levels of gamma radiation. An almost similar pattern of abundance was observed between the control and radiation treated samples.

Table 2. Variation in the relative abundance of SEED subsystem level 1 as determined by ANOVA and Kruskal-Wallis test. Of the 28 genes, only 3 had $P\text{-}_{values} > 0.05$ and they include cell division and cell cycle, respiration and secondary metabolism-related genes. The non-significant distribution of these genes implies their functions are affected by exposure to the ionizing irradiation. On the other hand, significant activities for stress-related genes were obtained along with DNA and RNA metabolisms suggesting ameliorative responses by the community to balance the effect of the ionizing radiation.

SEED Level 1 Gene	P value	X2 or F	DF
Amino acids and derivatives	**9.06e-07**	21.07	4,10
Carbohydrates	**0.000**	18.83	4
Cell division and Cell Cycle	0.472*	0.921	4,10
Cell wall and capsule	**1.96e-07**	25.68	4,10
Clustering based subsystems	**1.04e-05**	15.09	4,10
Cofactors, Vitamins, prosthetic group and pigments	**0.003**	15.61	4
DNA metabolism	**0.006**	5.155	4,10
Dormancy and sporulation	**7.48e-05**	11.25	4,10
Fatty acids, lipids and isoprenoids	**9.63e-05**	10.82	4,10
Iron acquisition and metabolism	**0.019**	11.82	4
Membrane transport	**5.18e-08**	29.91	4,10
Metabolism of aromatic compound	**0.002**	17.34	4
Miscellaneous	**1.65e-05**	14.12	4,10
Motility and chemotaxis	**5.18e-06**	16.38	4,10
Nitrogen Metabolism	**0.022**	11.50	4
Nucleoside and nucleotides	**1.57e-06**	19.58	4,10
Phages, prophages, transposable elements and plasmids	**0.001**	18.03	4,10
Phosphorus metabolism	**0.002**	6.345	4,10
Photosynthesis	**2.47e-05**	13.31	4,10
Potassium metabolism	**0.002**	16.65	4
Protein metabolism	**0.001**	7.638	4,10
Regulation and cell signaling	**0.023**	3.658	4,10
Respiration	0.151*	1.905	4,10
RNA metabolism	**0.048**	9.57	4
Secondary metabolism	0.743*	0.503	4,10
Stress response	**0.003**	6.06	4,10
Sulfur metabolism	**0.009**	4.641	4,10
Virulence, diseases and defense	**0.001208**	18.04	4

Key:* Non Significant $P\text{-}_{value}$ (at $P<0.05$); Significant $P\text{-}_{value}$ (at $P<0.05$) in bold

2.3.4. Microbial community and functional diversity

The diversity of all taxonomic groups and functional genes were significantly affected (at $P \leq 0.05$) in all the treatment levels (**Table A3**). The species diversity decreased with increasing radiation intensity but interestingly, gene functional diversity (at SEED level three) increased (**Figure 7**). The species evenness also decreased with increasing radiation dose, but the species richness increased with increasing radiation (**Figure A5a** and **b**). Functional gene richness also increased with increasing irradiation but the evenness of functional genes showed no significant difference (**Figure A5c** and **d**).

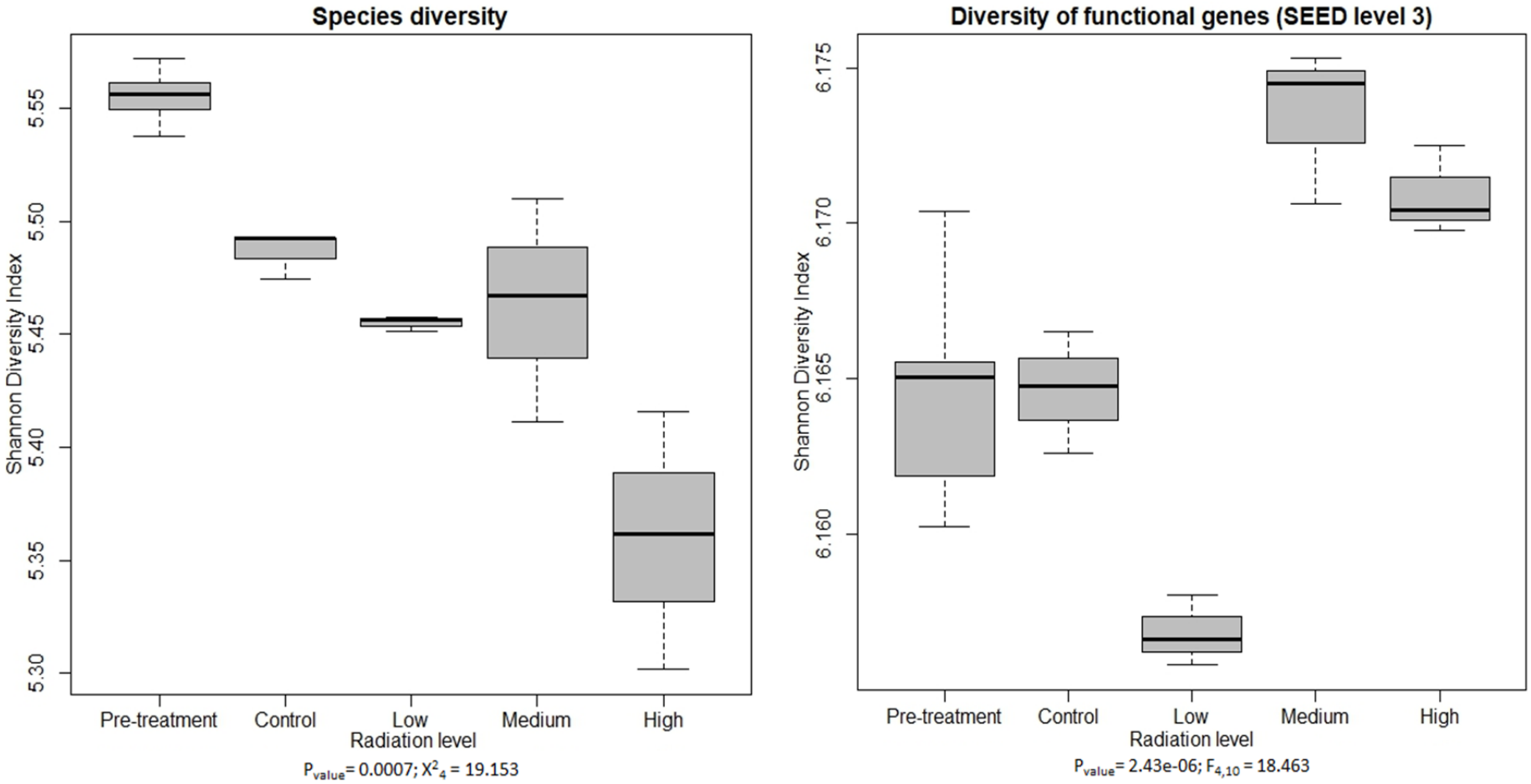


Figure 7. Shannon diversity index of species and functional genes suggest that with increasing radiation species diversity decreases. Functional diversity increased under medium and high irradiation but decreased under low radiation treatment. These were also significantly different at $p \leq 0.05$

These effects were further analyzed at the family level, which revealed that algal diversity increased with increasing radiation intensity, whereas bacterial and metazoan diversity decreased with increasing radiation intensity (**Figure 8a - c**). Fungi diversity also increased significantly in response to radiation, although not as strong as the response of algae (**Figure 8**).

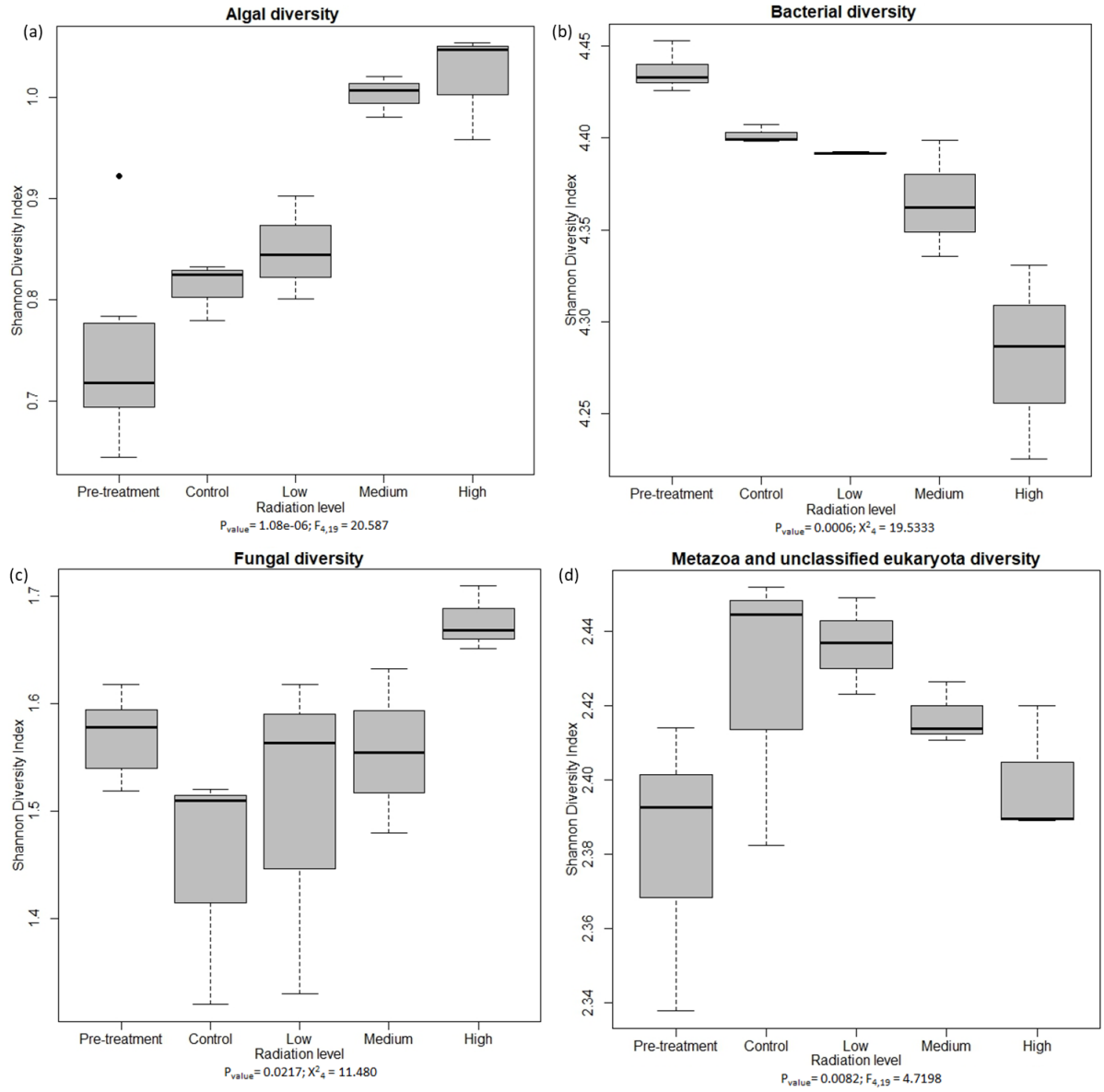


Figure 8. Shannon diversity index for algae (**a**), bacteria (**b**), fungi (**c**) and metazoa (**d**) families reveal alternating patterns in response the radiation treatment. They were all significantly diverse. For algal families, with increasing radiation intensity, their diversity increased. Fungal diversity was highest under high ionizing radiation intensity. As the intensity of the ionizing radiation increased, bacteria and metazoan diversity decreased.

In order to understand the effects of the irradiation treatments on functional diversity within selected categories of genes, SEED level 3 was assessed further. The results varied by gene category, for example, the diversity either decreased, increased or showed inconsistent patterns with increasing radiation dose (**Figure 9a - f**).

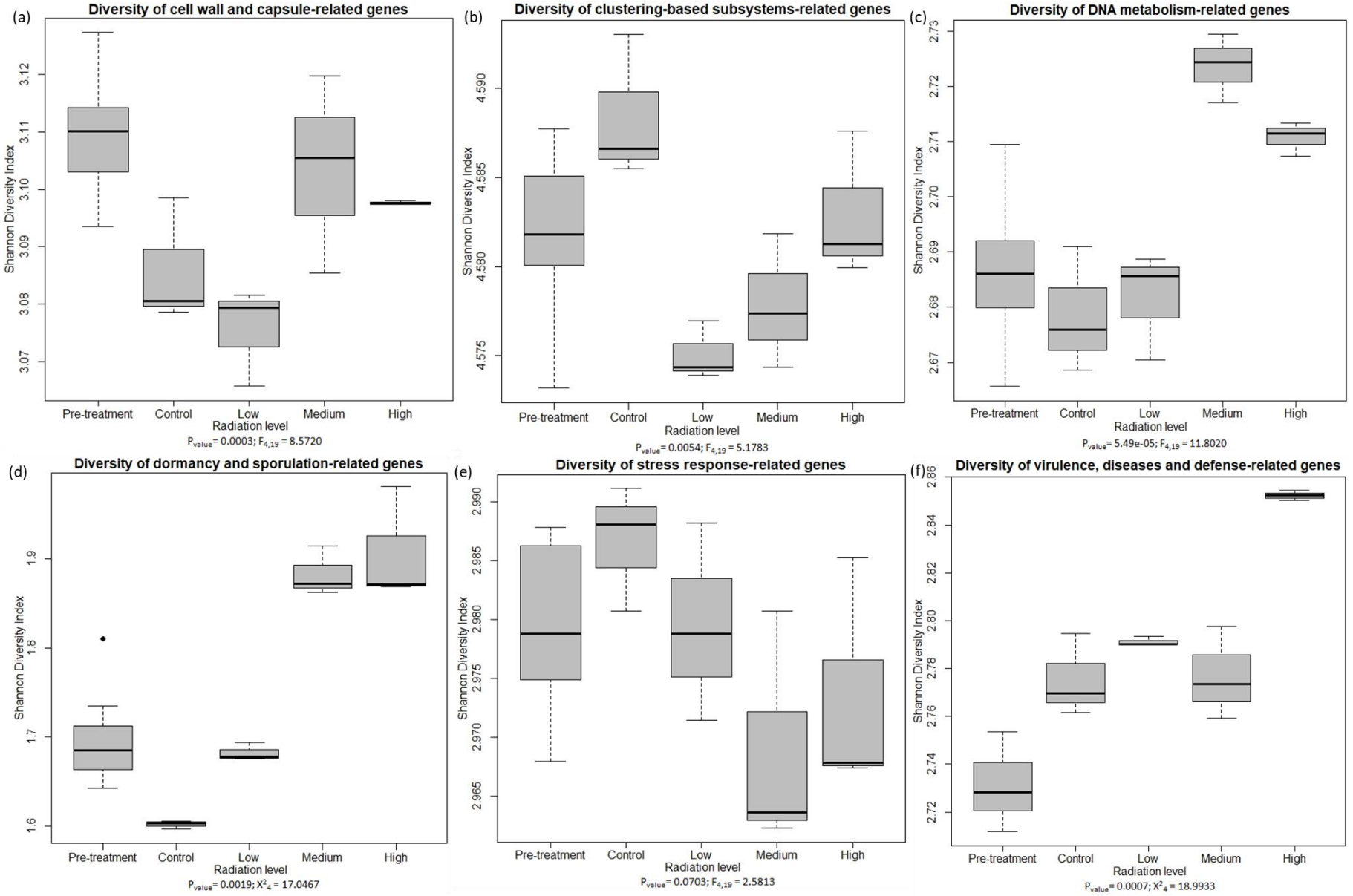

(a)
Diversity of cell wall and capsule-related genes
Shannon Diversity Index
3.07
3.08
3.09
3.10
3.11
3.12
Pre-treatment
Control
Low
Medium
High
Radiation level
P_{value}= 0.0003; $F_{4,19}$ = 8.5720
(b)
Diversity of clustering-based subsystems-related genes
Shannon Diversity Index
4.575
4.580
4.585
4.590
Pre-treatment
Control
Low
Medium
High
Radiation level
P_{value}= 0.0054; $F_{4,19}$ = 5.1783
(c)
Diversity of DNA metabolism-related genes
Shannon Diversity Index
2.67
2.68
2.69
2.70
2.71
2.72
2.73
Pre-treatment
Control
Low
Medium
High
Radiation level
P_{value}= 5.49e-05; $F_{4,19}$ = 11.8020
(d)
Diversity of dormancy and sporulation-related genes
Shannon Diversity Index
1.6
1.7
1.8
1.9
Pre-treatment
Control
Low
Medium
High
Radiation level
P_{value}= 0.0019; X^2_4 = 17.0467
(e)
Diversity of stress response-related genes
Shannon Diversity Index
2.965
2.970
2.975
2.980
2.985
2.990
Pre-treatment
Control
Low
Medium
High
Radiation level
P_{value}= 0.0703; $F_{4,19}$ = 2.5813
(f)
Diversity of virulence, diseases and defense-related genes
Shannon Diversity Index
2.72
2.74
2.76
2.78
2.80
2.82
2.84
2.86
Pre-treatment
Control
Low
Medium
High
Radiation level
P_{value}= 0.0007; X^2_4 = 18.9933

Figure 9. Shannon diversity index of SEED level 3 functional genes including cell wall and capsules (**a**), clustering based subsystems (**b**), DNA metabolism (**c**), dormancy and sporulation (**d**), stress response (**e**) and virulence, disease and defence-related genes.

Cell wall and capsule-related genes were most diverse in the pre-treatment samples closely followed by medium treated samples (**a**). In clustering based subsystems, the Shannon diversity index was highest in the incubated samples (Control) with diversity increasing as radiation intensity increases (**b**). Furthermore, the highest Shannon diversity index was obtained from the medium, high radiation treated samples in DNA metabolism (**c**) and dormancy, and sporulation-related genes (**d**). In stress response-related genes, the Shannon diversity index was lowest in medium and high radiation treated samples (**e**) but highest in the high radiation treated sample for virulence, disease and defence-related genes (**f**).

To visualize the degree of overall similarity in soil biota taxonomic composition or functional gene composition between the treatment levels, after computation of differences in relative abundance of the different treatment levels, results were ordinated using NMDS. For the taxonomic perspective, the NMDS ordination was generated at the species level (**Figure 10a**) and for functional genes at level four of SEED subsystems (**Figure 10b**). The clustering pattern of samples indicated distinct and repeatable taxonomic and functional genes composition of the soil biota for each treatment. Microbial community and functional diversity co-varied and positively correlated with one another.

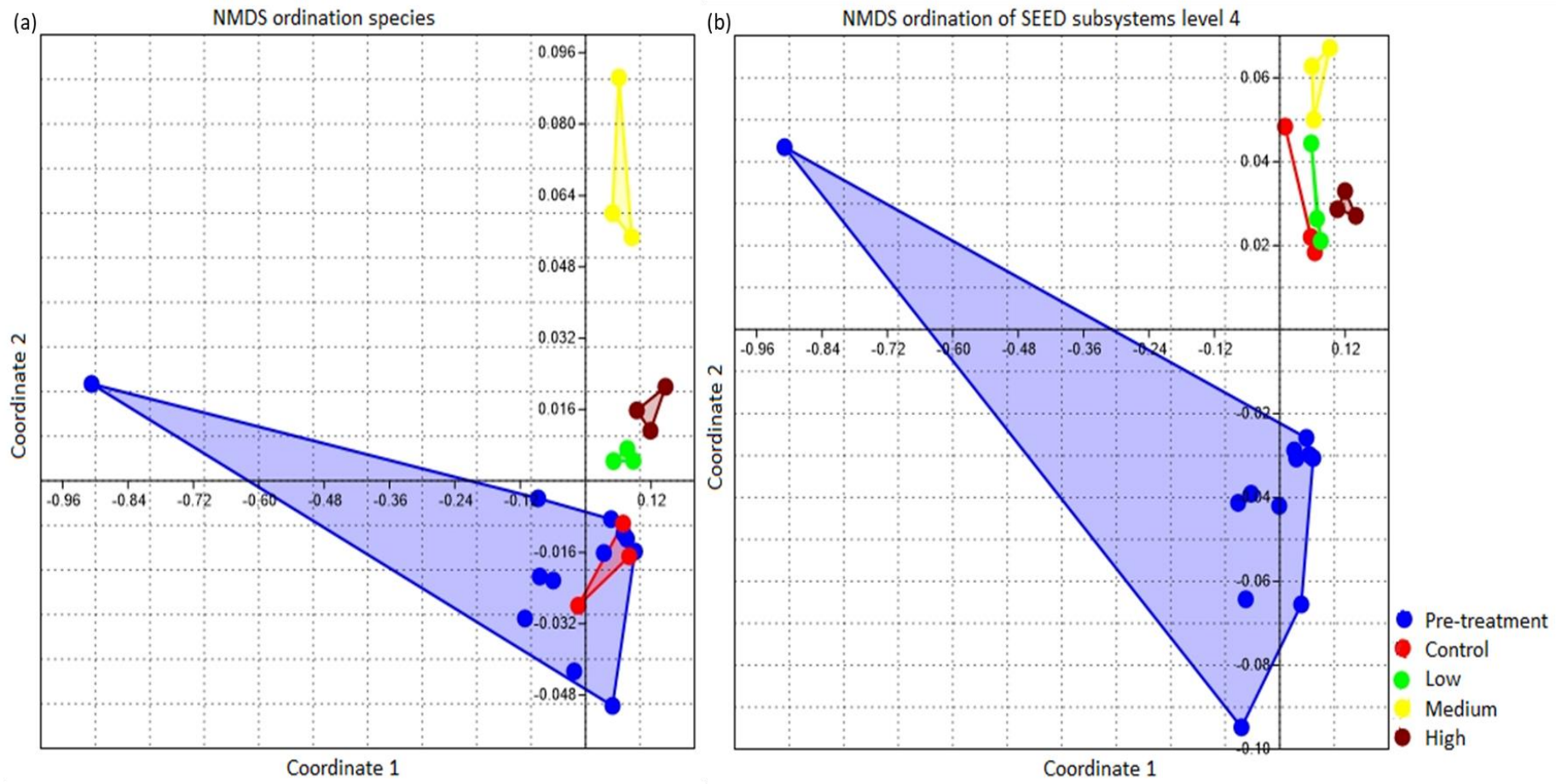


Figure 10. NMDS ordination of species (**a**) and SEED subsystems level four genes (**b**). Radiation treated samples clustered close to each other suggesting more similarity within the group than between groups.

2.4. Discussion

The metagenome approach used in this study enabled us to assess the effects of repeated exposures of ionizing radiation on microbial diversity and functional gene composition of soil microbial communities. The results reveal soil biota community changes, which themselves may have influenced soil chemical characteristics, such as reduced retention of soil TP and TN with radiation exposure (Table A1). This supports the conclusion of Wagg et al. (2014) that the disturbance of soil microbial diversity and composition causes a reduction in multiple ecosystem functions.

Effects of γ-Irradiation on Soil Chemistry.

Soil chemical analysis showed that the irradiated soils differed chemically in certain aspects from the untreated controls (**Table A1**, **Figure A1**). However, it is unclear whether this is a direct effect of the radiation or an effect of changes in the soil biota. If different groups of organisms become dominant after radiation exposure, their differing metabolic activities might alter the soil chemistry. Additionally, dead microorganisms are capable of producing legacy interactions in the soil through their DNA and enzyme actions. The use of γ-irradiation in soil sterilization may kill microbes but not inactivate their enzymes, which could leave a legacy on soil matter decomposition through continued activities (Blankinship et al., 2014). Thus, the observed changes in soil chemistry between treatments could be partly due to these effects, which is similar to the observation of Jurburg et al. (2017) in a microcosm experiment subjected to extreme temperature perturbations.

Irradiation of soil is capable of influencing complex chemical processes through alterations to nitrogen availability, changing the pH and phosphate concentration in the soil (Eno and Popenoe, 1962; Wainwright et al. 1980; Singh, 1997; Rejsek et al. 2010; Iheme et al., 2016). The observed changes in TN with increasing γ-radiation intensity may have affected

the abundance of certain taxa and functional genes. In this study, the lack of any large changes in pH, even from very high doses, agrees with the results of Bank et al. (2008). Together, these results suggest that soil pH may be resistant to change due to γ-irradiation.

Differential leaching is presumably responsible for the difference in TP concentration between pre-treated samples and the other samples (due to weekly watering) but not across treatment difference. Changes in TOC in the study can be attributed to the disintegrating effects of the ionizing radiation on carbon (hypothetically rendering them more soluble and leachable, or available for catabolism), or death of living biomass and subsequent catabolism of these cells by the surviving populations of microbes. This, in turn, could have affected microbial activities and community structure by resulting in a shift in their energy utilization pattern. Changes in soil chemistry can interfere with both biotic and abiotic processes (Berns et al., 2008). Soil chemicals are important for the normal functioning of the soil microbiome and higher life forms such as plants that require them in balanced concentrations. For instance, the abundance of bacterial and fungal heterotrophs within a decomposer community correlates with their metabolizing functions, substrate pool and soil quality (Fabian et al., 2017). Therefore, the depletion of these substrates may, in turn, affect their abundance and overall diversity. As observed in this present study, an opportunistic group like algae may use the opportunity to increase their diversity (**Figure 7**).

Hypothesis 1. Soils exposed to γ-irradiation will have a taxonomically distinct biota with lower diversity compared to untreated control samples.

This hypothesis was supported by the reported results. For all the major groups of organisms, radiation exposure selected a very distinct soil community. For bacteria (**Figures 3**, **A2b** and **A3a**), comparing the control and radiation-treated soils, there was a shift from Proteobacteria (in the no-radiation controls) to Deinococcus-Thermus (which increased with

increasing radiation treatments). The ability of Deinococcus-Thermus to resist high doses of ionizing radiation is well known, although the community context in which it becomes more abundant has not been investigated before. *Deinococcus* species are known for their tolerance of very high doses of radiation because of their high DNA repair capacity and resistance to oxidative damage (Battista, 1997; Battista et al., 1999; Nelson et al., 2000; Makarova et al., 2001; Daly et al., 2004; Daly, 2009). The absence of this complex DNA repair system in other groups such as Actinobacteria and Chlamydiae may leave them more vulnerable to irradiation. However, Chloroflexi, which also lack the same mechanisms as in Deinococcus-Thermus, increased in relative abundance in the irradiated treatments – suggesting that they may be adopting a different strategy to resist γ-irradiation effects. They appear to represent a previously unidentified potential group of radiation resistors, whose adaptations to survive radiation merit further study.

For archaea, although their relative abundance remained low (~ 1 % of the total reads), there was a striking shift in phyla composition, from mainly Euryarchaeota in the control samples to a community co-dominated by Crenarchaeota, Thaumarchaeota, and Nanoarchaeota (**Figures 4b** and **A2b**). These latter groups – especially Nanoarchaeota - include known extremophiles noted for their tolerance of high temperature and acidic environments, but not as radiation resistors. Nanoarchaeota lack DNA polymerases and histone proteins, along with various other genes, which are present in Euryarchaeota (Brochier et al., 2005). Whether these differences confer radiation-resistance is unclear.

Amongst fungi, radiation exposure brought about a shift from a community dominated by Ascomycota to one dominated by Chytridiomycetes, and Basidiomycota (**Figure 4c**). This contrasts with the results of simulated space conditions and radiation treatments, where Ascomycota became dominant due to the presence of melanin (Revankar and Sutton, 2010; Blachowicz et al., 2017) for irradiation energy capture and utilization (Dadachova et al., 2007).

However, soil Ascomycota is generally viewed as rapidly growing forms which exploit nutrient-rich substrates (Pugh, 1980), and their rapid pace of DNA replication may have made them more susceptible to radiation damage. Their reduction in abundance observed here may have presented an opportunity for the even faster growing Chytridiomycota to exploit the dead cellular materials left after each radiation exposure – even if the temporary effects of radiation on their populations may be expected to be more severe. By contrast, the increased relative abundance of Basidiomycota might be related to their slower growth rates, on recalcitrant substrates (Pugh, 1980; Colpert and Tichelen, 1996) considering the break-burst irradiation approach adopted in this study. Moreover, if low rates of DNA replication make their cells less susceptible to irradiation damage, then this may explain their preferential survival in the soil.

As anticipated, total species diversity decreased with increasing radiation intensity (**Figure 7**), including the most abundant taxonomic domain, bacteria (**Figure 8**). Thus, it appears that high doses of ionizing radiation have a strong influence on the taxonomic composition of the soil (**Figure A5**). However, it is unclear if and when the diversity of a radiation-disturbed disturbed system would return to its pre-disturbance state, and what role local evolution of resistant strains might play in this (McNamara et al., 2007). Shannon diversity of metazoan families also decreased significantly compared to the control. However, in contrast to set hypothesis, the diversity of fungal and algal (eukaryote) families increased significantly, especially at the highest intensity of γ-irradiation (**Figure 8**). Previous results by McNamara et al. (2007) suggested that eukaryote (fungal) populations do not recover substantially after irradiation, this finding is supported by the decreased fungal absolute abundance (from ITS copy number in qPCR, **Figure 5**), even though diversity was greater. These diversity changes may imply that either change in niche competition structure or a change in nutrient availability favoured fungal diversity. At least some fungi possess radio-protective melanin, which may even harvest energy for the cell and allow them to increase

growth after exposure to ionizing radiation (Dadachova et al., 2007; Dadachova and Casadevall, 2008). Fungal communities have been reported in radiation spill sites after prolonged periods and even grow towards warm highly radioactive particles (Zhdanova et al., 2004).

Overall, it appears that most major taxonomic and ecological categories of soil organisms have at least some representation in even the most strongly irradiated samples. Lignin and cellulose decomposing taxa, ammonia oxidising archaeal and bacterial taxa, N fixing taxa, etc. as well as microbe-grazing protists and metazoans, are all still present in the highest radiation dose treatments. What has evidently changed is the overall biomass of these groups (according to qPCR of copy numbers of bacteria and fungi (**Figure 5**)), and their relative abundances (see discussion on Hypothesis 3, below). How this might affect ecosystem function and soil sustainability remains unknown, and a topic for future work.

Hypothesis 2. Soils exposed to γ-radiation will have a lower diversity of functional genes.

In contradiction to the hypothesis, a greater diversity of functional genes was observed at higher doses of ionizing radiation (**Figures 7** and **9**). In addition, the diversity of many subcategories of functional genes increased significantly, including those associated with cell wall and capsule, dormancy and sporulation, regulation and cell signalling, stress-related genes, virulence, diseases and defence (**Figure 9**). This may be an indication that additional functions are required to survive stress from ionizing radiation. While radiation-induced mutation can increase low-level gene sequence diversity (Hanafiah et al., 2017), an increase in community-level gene function diversity has not been reported before. Moreover, certain taxa may use these genes to recover and grow quickly from irradiation while there is little competition and abundant resources (dead cells).

The increase in gene function diversity runs in opposition to the decreasing total taxonomic diversity of the system. This supports the suggestion of Souza et al. (2016) that taxonomic diversity may not necessarily be associated with functional diversity. Although in a stress-altered system, functional acclimatization is necessary, it is often costly and results in community shift or resource reallocation (Schimel et al., 2007). How changes in functional gene diversity influence the overall community functioning is unclear and would require empirical measures of soil functions such as catabolic diversity to explain (Petchey and Gaston, 2002; Torsvik and Øvreås, 2002). This may also require higher doses of γ-irradiation to initiate significant metabolic changes (Stotzky and Mortensen, 1958). However, it is worth noting that differences between radiation-treated samples provide insights into the intensity of radiation effects on microbial functional diversity.

Hypothesis 3. Changes in abundance of certain groups of genes associated with radiation exposure.

It was hypothesized that there would be an increase in the relative abundance of stress response genes, an increase in dormancy and sporulation-related genes, and a decrease in genes related to competition and cell-cell interactions, following γ-irradiation (**Figure 9**). In spite of the observed pattern, the presence of these genes may not be related to their functionality. In addition, their expression by the parameters in this study may be in co-occurrence with other functions. An organism that invests in recovery following a disturbance also carries many functional genes that would allow it to perform key functions such as energy generation in the new modified environment as well as provide necessary components to be successful like nitrogen fixation.

However, gamma irradiation interferes with the biochemical processes of microorganisms, to produce an array of outcomes (Eno and Popenoe, 1962; Schimel et al.,

2007). Microbial stress response mechanisms are species-specific and diverse (Sévin et al., 2016). Therefore, members of the community in the studied system may be using different tolerance mechanisms in relation to γ-radiation stress, beyond those stress response genes identified in MG-RAST. Riley (1993) reported that oxidative stress-related genes are required to act upon the hydroxyl radicals generated by ionizing radiation. Dormancy-related genes may bring about growth retardation under stress, aiding survival (Mlynárová et al., 2007). This prediction was true in the study as the relative abundance and diversity of dormancy and sporulation genes increased with γ-radiation intensity (**Figure 9**). These genes increased with increasing intensity of ionizing radiation, even though spore-forming groups of Proteobacteria and Cyanobacteria did not increase in abundance under irradiation. Organisms with significantly increased levels of relative abundance in the study, including Deinococcus-Thermus and Chloroflexi have been known to express high levels of dormancy under adverse unfavourable conditions (**Figures 3** and **A3a**). After dormancy, functionality may not be affected as during dormancy, spores of radio-resistant and dessicophilic microorganisms accumulate DNA damage. Spores lack water and thus are metabolically inactive, with no DNA repair possible until germination (Mattimore and Battista, 1996; Pointing et al., 2007; Dartnell et al., 2007). Therefore, the abundance of dormancy and sporulation-related genes may increase the chance of survival when irradiated. The normal ecological processes governing abundance and diversity may not be easily explicable when microorganisms enter dormancy under ionizing radiation (Wei et al., 2016) or other disturbances.

However, in contradiction to another aspect of the set hypotheses, following irradiation, the abundance and diversity of virulence genes increased (**Figure 9**). Since these genes are important in positive and negative biotic interactions within the soil, this suggests a greater importance of interference competition regulating community structure under disturbance from ionizing radiation. It seems then, that under radiation, cell-cell interactions may be important

to survival – in contrast to their prevailing view that stressful environments involve less biotic interaction.

The abundance of viruses and transposons increased in the treated samples, possibly suggesting a breakdown of defense mechanisms in radiation-stressed cells and increased vulnerability to viral attack. Likewise, CRISPR genes – involved in defense against viruses – increased, suggesting greater selection on bacteria from viral attack. Another possibility is that mutation promoted by γ-irradiation acts more effectively in increasing viral genetic diversity, at the expense of bacterial populations, which are unable to evolve defences as quickly.

2.5. Concluding Remarks

In conclusion, a range of different changes can be found in the soil system following repeated large doses of γ-irradiation. Importantly, total bacterial and fungal biomass in the irradiated soils was much lower than the untreated soil, which implies the capability of the soil system to carry out ecosystem functions remains greatly impaired by irradiation. One empirical indicator of changes in ecosystem functions is the greater TP concentration in the irradiated soils as compared to the control soil after six weeks. This may indicate that TP in the soil remains more stable in the face of leaching with the overall loss of biomass and soil community changes associated with radiation, with implications for predicting loss of radionuclides or toxins from irradiated soils. By contrast, however, TN and TC are lower, implying that they become more labile and more easily lost from the soil system = or in the case of N, less easily replaced.

The most surprising result of this study was an increase in the finer level (level 3) functional gene diversity and in the taxonomic diversity of selected groups of organisms (fungi, and algae). It is unclear why repeated cycles of radiation exposure would produce an increase in taxonomic and functional diversity. Such increases in diversity may provide an example of the principle of 'competitive release' seen in studies of sedentary large organisms (Huston 1993), whereby a certain amount of mortality can increase taxonomic diversity. However, these were only increases in taxonomic diversity: an increase in trait diversity has never been noted for larger organisms. More so, it is likely that the high resource availability (from all the dead microbes) could have created a greater number of niches with irradiation even with uneven nutrient availability. Hence, with the decimated micro population (and environment), recovery will have unevenness and stochasticity that can contribute to diversity especially if the next round of irradiation occurs before a stable community is reached.

The increased trait diversity seen in the irradiated soils may be expected to increase the versatility of the biota in the soil ecosystem – for example in metabolizing pollutant metals associated with radioactive waste – although overall living biomass from qPCR is lower and this in itself might impair soil ecosystem functions. The study also pinpointed novel examples of radiation-tolerant groups of organisms: for example, the Nanoarchaeota (archaea), and Chytridiomycota (fungi). Possibly some of these can remain active through radiation exposure, whereas others are able to survive in dormant form and then increase rapidly to exploit the aftermath. Further investigation of the radio-tolerance mechanisms of these groups should be a high priority in future studies.

Some caveats need to be underscored as a possible theme for further work. For example, in this study, only one soil was investigated. As this is apparently the first instance in which a soil has been investigated metagomically in this context, it is unclear how other soils might respond to radiation exposure from the whole biota perspective. However, one might expect that in a less nutrient-rich but organically rich soil, or in a colder environment, the recovery rate of the soil biota between radiation phases would be slower, perhaps with lower taxonomic diversity as fewer forms would be able to maintain their population levels. It is interesting to speculate that in drier or chemically more extreme soil environments, the biota might be adapted (in terms of taxa present or genes) to radiation resistance (Goberna et al., 2014). Moreover, as observed most metagenome study (Delmont et al., 2011), the soil in this study contained higher levels of prokaryotic than eukaryotic diversity but functional interpretations depends on recovered DNA from the soil. The microbial composition and functions of the pre-treatment and control soils in this study are within the scope of those in reported in other works with similar support functions (Castenada and Barbosa, 2017; Jansson and Hofmockel, 2018; Feng et al., 2018). In a similar way, the irradiation treatments give a clear picture of what may be anticipated (i.e. successional consequences) under environmental extremes (like in the

Antarctic and elsewhere) as well as in a soil that is contaminated as reported in Rainey et al. (2005); Brown et al. (2015); Koo et al. (2018). Chemically, the soil is also representative of the Korean soil in TOC (Hwang et al., 2000; Hong et al., 2010; Igalavithana et al., 2017).

To more rigorously eliminate the possibility that some of the results were derived from 'legacy' DNA from cells that died during radiation exposure, analysis of cDNA or use DNase digestion or propidium monoazide will be required. The latter has also been documented to improve the detection and distinction of living and dead cells in PCR (Nocker et al., 2007).

Lastly, the study was constrained to running experiments with repeated bursts of radiation exposure. Study under continuous radiation exposure, spanning months or ideally years, might provide findings more relevant to the long-term impacts of contamination on sites localities or even cumulative effects of radiation in space biology.

PART 3. COMMUNITY ECOLOGY OF A GAMMA-IRRADIATED DEINOCOCCUS DOMINATED SOIL

3.1. Introduction

Most work on the effect of ionizing radiation exposure on microbes has dealt with cultures, either pure or mixed cultures (Stroes-Gascoyne et al. 1994; Lucht and Stroes-Gascoyne, 1996; Shuryak et al., 2017, Wright and Hill, 1968, Jeon et al., 2016), and the effects of radiation on soil bacterial communities are relatively unexplored. Among the few reported radioecology soil community-based studies, bacterial diversity and richness reduced with increasing ^{60}Co gamma irradiation (Popenoe and Eno, 1962; Rainey et al., 2005; McNamara et al., 2007). Brown et al. (2015) reported that after eight weeks of continuous 30-Gy/hr gamma-irradiation, the communities were dominated by *Clostridia* sp. and *Geobacter* sp. while *Geothrix fermentans* dominated sediments irradiated at 0.5-Gy/hr showed a significant increase in fermentation and iron reduction-related genes as determined through a combined culturing and 16S rRNA gene sequencing approach. Using the same approach at doses of 17 – 30 kGy (at a dose rate of 2.57 kGy/h) of gamma irradiation, Rainey et al. (2005) reported the recovery of *Deinococcus, Geodermatophilus* and *Hymenobacter* in culture. In another study using culture-independent denaturing gradient gel electrophoresis analysis of 16S rRNA gene fragments, El-Sayed and Ghanem (2009) suggested that Actinobacteria, Alphaproteobacteria, Gammaproteobacteria, Chloroflexi, *Enterobacter* and *Pseudomonas* were all relatively resistant to irradiation.

Small doses (up to ~0.000001 Gy) of ionizing radiation alter the pre-existing balance of relative abundances of microbes within the soil community (Monib and Zayed, 1963). Larger doses have clearer qualitative impacts on the soil microbial community, although complicated by the legacy effects of dead cells and their residual enzyme functions, as well as induction of the release of breakdown products of organic molecules (McLaren, 1969; Romanovskaia et al., 2002; El-Sayed and Ghanem, 2009; Brown, 2013; Brown et al., 2017). This effect depends on the soil type and its physicochemical properties (McLaren, 1969). No

previous studies have attempted to combine metagenetic and metagenomic techniques in next-generation sequencing to consider in detail the *in situ* bacterial community structure and functions after irradiation.

It is well established from culturing studies (Venkateswaran et al., 2000; Sukhi et al., 2009; Bornot et al., 2015) that the genus *Deinococcus* (from the phylum Deinococcus-Thermus [DT]) is exceptionally resistant to radiation, being able to survive at doses up to 30 kGy (Rainey et al., 2005). However, there have been no studies on the community ecology of this genus in highly irradiated soils, in terms of diversity, coexistence of species and relative abundances. In the context of the overall soil bacterial community, this study will also investigate the role that *Deinococcus* may play in this, and the implications for potential ecosystem properties of the whole community.

The study of irradiated soils is a vital component in understanding the effects of radioactive waste spills on soil biology and processes as well as in the context of predicting the resilience of soil function and effects on mobilization of radionuclide ions (Ragon et al., 2011; Ruiz-González et al., 2016; Castillo and Smith, 2017; Repar et al., 2017). It is also of interest in the context of maintaining soil systems in space travel, and on the surface of other planets, where radiation exposure can be high without the shielding effect of an Earth-like atmosphere and magnetic field (Mattimore and Battista, 1996; Rainey et al., 2005). If biological introduction of Earth-derived life forms to other planets is a concern, understanding the diversity of irradiated soils may also help to predict what forms are most likely to arrive accidentally through high radiation environment in deep space, and what sort of soil systems they might form if artificially introduced to other planets with higher radiation fluxes. Analysis of the functional ecology of irradiated soil bacterial systems may also give guidance to the requirements for genetically manipulating or treating organisms to resist radiation exposure in

order to form viable soil systems (Saito et al., 1994; Asgarani et al., 2000; Liu et al., 2003; de la Torre et al., 2003; Zhdanova et al., 2003; Robinson et al., 2011).

The main questions that this study was interested in answering include:

1) How does the community structure of Deinococcus change with increasing radiation exposure, and do different Deinococcus forms dominate at different radiation intensities – suggesting the existence of 'radiation niches'?

It is unclear from previous radiation studies, which were mostly culture based, what the structure of soil *Deinococcus* communities is. For example, whether multiple species of *Deinococcus* become co-dominant, or whether certain individual species become dominant. It is also unclear whether a pre-adapted niche differentiation emerges when different doses of radiation are used - for example with some *Deinococcus* being particularly abundant in certain ranges of radiation dose intensity.

2) What features within the genomes of Deinococcus predominating at higher radiation exposures might confer greater 'success', compared to the Deinococcus community predominating at low radiation exposures?

Using 16S taxonomic data from amplicon and metagenome sequencing, We hope to identify the *Deinococcus* species present as known species whose genomes have already been published. This would allow us to infer why some species might be more abundant than others – in terms of radiation damage, repair systems, quorum systems or trophic capabilities. This could give important indications to the key features of the genus that are important in the competitive success of *Deinococcus* in irradiated soil systems. We also aimed to compare the entire profile of *Deinococcus*-annotated genes present in the soil metagenome between the different treatments, to understand the traits within members of this genus, which are

ecologically selected by high radiation exposures. The overall picture gained from these analyses can potentially increase understanding of the most important features necessary for survival under high radiation exposures.

3) Are there other groups of bacteria, which increase in relative abundance in response to irradiation?

Apart from the well-known *Deinococcus*, what other bacteria groups (whether these are known already as radiation resistors or novel species) would become relatively more abundant under radiation exposure, forming a significant part of the irradiated soil community?

4) What does the profile of functional genes present in the total soil metagenome – and in the known published genomes of Deinococcus and other dominant bacteria present – indicate about the functional capabilities and resilience of the irradiated soils?

Based on the functional gene profile, what ecosystem capabilities exist and predominate in the irradiated soil? For instance, are genes for the breakdown of lignin and cellulose relatively abundant? Are there genes for N fixation or other key aspects of the nitrogen cycle? What are the general features of the soil system – in terms of the breakdown of organic matter, mobilization of ions, etc., and how does it differ from the control soil/other non-irradiated soils?

3.2. Materials and Methods

3.2.1. Sampling, Soil Collection and DNA Extraction

The soil sampling method, irradiation treatment and autoclaving conditions were the same as those reported in Part 2 (see section **2.2.1** and **2.2.2**). Immediately after six weeks of gamma irradiation treatment, three samples were collected from each pot (of Controls and, Low, Medium and High) for total genomic DNA extraction and PCR (also as enumerated in Part 2 (section **2.2.4**)). DNA from three pre-treatment soils was extracted soon after collection and stored at -20 ^{o}C.

3.2.2. Bacteria 16S rRNA Pyrosequencing and Sequence Processing

Extracted DNA was sent for sequencing at the Graduate School of Public Health, Seoul National University, Republic of Korea using the Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA). The sequencing was performed according to the manufacturer's instructions as presented in Dong et al. (2017) and is summarized as follows: the 16S rRNA amplicon paired-end sequencing (2 x 300 bp) focussed on the small fragments of the V3-V4 hypervariable region of the 16S rRNA gene. By titrating the concentrations of 16S rRNA gene amplicons to the flow cells and using a quality score-based approach to correct discrepancies between reads used to construct contigs and reduce errors. Initially, the primer pair sequences for the region (5' (i.e. forward 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and reverse 5'-' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATC C-3') was generated into a single amplicon library. Then a paired 300bp reads and Miseq reagents were used to generate high quality, full length reads of the region in a single 65-hour run. The fastq Miseq sequences outputs are deposited in the NCBI database with accession number SRA158005.

The Miseq sequence data were processed using Mothur platform (version 1.32.1, http://www.mothur.org) (Schloss et al., 2009). The sequences were aligned using Mothur (default settings: kmer searching with 8mers and the Needleman–Wunsch pairwise alignment method). Next, they were aligned against the EzTaxon-aligned reference (Chun et al., 2007), and further filtered to remove gaps. Sequences were de-noised using the '*pre.cluster*' command in Mothur implementation of pseudo-single linkage pre-clustering algorithm from Huse et al. (2008). Putative chimeric sequences were detected and removed via the Chimera Uchime algorithm contained within Mothur (Edgar et al., 2011) in *de novo* mode, which first splits sequences into groups and then checks each sequence within a group using the more abundant groups as a reference. The taxonomic classification was performed using Mothur's version of the RDP Bayesian classifier, using EzTaxon-e database for each sequence at 80% Naïve Bayesian bootstrap cut-off with 1000 iterations.

The OTUs were used for alpha diversity, richness and abundance calculation including Shannon, Chao, Abundance-based Coverage Estimator [ACE], Inverse Simpson, Broken-stick, Log and Geometric series as well as to generate a Rarefaction curve, in Mothur.

3.2.3. Statistical Analysis

The relative abundance was used to determine the most abundant taxa within and between each treatment condition. The level of significance of their abundance was determined in R (http://www.r-project.org/ [version 3.4.0] R Development Core Team, 2008). Shapiro-Wilk's normality test was first used to determine their distribution and depending on their normal or non-normal status, I subjected them to ANOVA (and Tukey HSD posthoc) or Kruskal-Wallis (and Pairwise Wilcox) test. The relative abundance was used to generate heatmaps using the pheatmap package in R. Non-metric multidimensional scaling (NMDS), was used to visualize the community structure using Primer-E software (Version 6, Plymouth,

UK) by using Bray-Curtis index for taxa and OTU-based community similarity and Euclidean distance for environmental parameters, which where fitted into the analysis.

OTU alpha diversity and abundance of indices were calculated using the Mothur platform-using summary. single command while those of taxa were calculated using the vegan package in R (Oksanen et al. 2013).

The NCBI BLAST of community sequences was done as enumerated in Tao (2010) to find out the major *Deinococcus* representatives. Functional annotation of Deinococcus-Thermus and the whole bacterial community was carried out using available sequence data on MG-RAST server (Meyer et al., 2008) with the project ID 20322 using the filtering function for SEED subsystems-related genes. Comparative genome analysis of the most abundant *Deinococcus* species was done using uploaded sequences on NCBI including PRJNA16691 (*Deinococcus deserti* VCD115), PRJNA188857 (*Deinococcus ficus* DSM11300), PRJNA13423 (*Deinococcus geothermalis* DSM11300), PRJDB4362 (*Deinococcus grandis* ATCC43672), PRJNA188858 (*Deinococcus murrayi* DSM11303) and PRJNA315481 (*Deinococcus radiodurans* ATCC BAA-816). The transmembrane proteins of the most abundant *Deinococcus* species were predicted using the Transmembrane Membrane Protein Hidden Markov Model Server, v. 2.0 (Krogh et al., 2001) while the carbohydrate-active enzymes (CAZymes) were predicted using the dbCAN-seq server (Huang et al., 2018). The genomes were also subjected to Rapid Annotation using Subsystems Technology [RAST] (Aziz et al., 2008) using their online server.

3.3. Results

The total number of quality sequences obtained from the samples was 1,066,251 (containing 130,361 unique sequences) with an average length of 450 bases, which were classified into 6,271 operational taxonomic units (OTU's) at a 97 % similarity level after rarifying. The rarefaction curve showed a clear variation in the numbers of OTUs detected versus number of sequences analysed among the radiation treatment levels (high and medium) while pre-treatment, control and low levels were not significantly different (**Figure 11**).

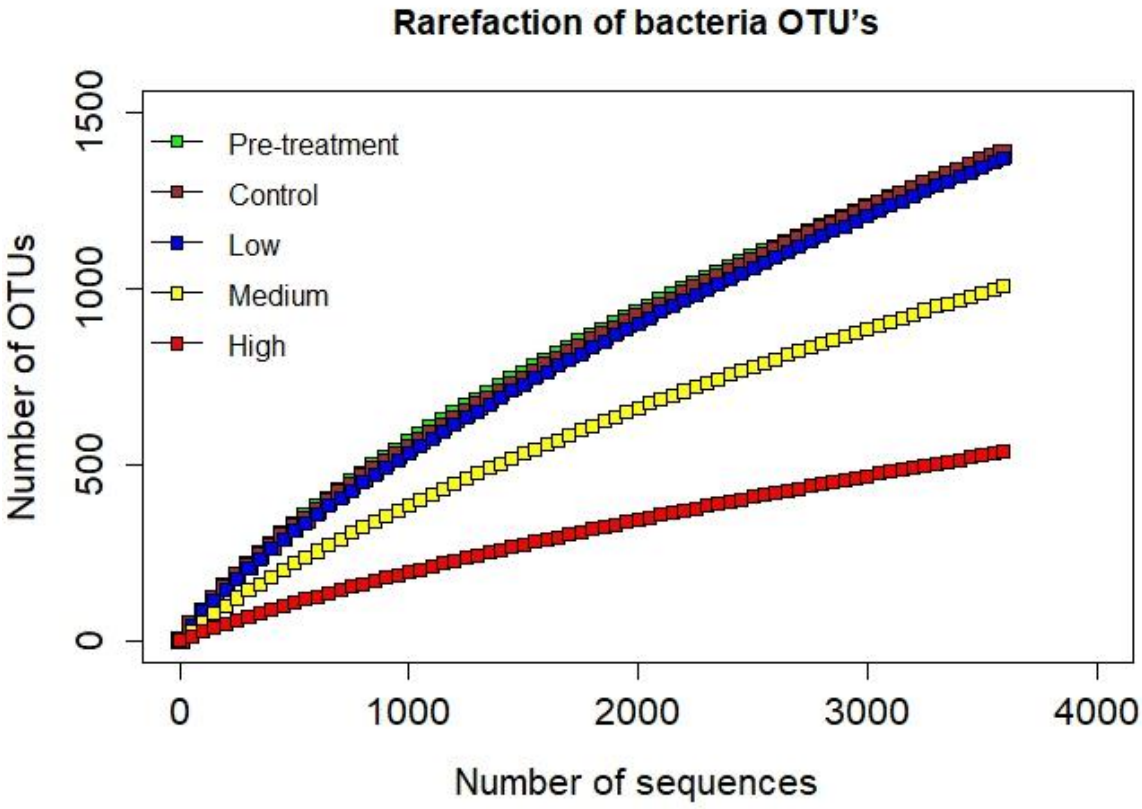


Figure 11. OTU rarefaction analysis of soil bacteria 16S rRNA genes of the samples calculated using 0.3 OTU definition (at 97 % sequence similarity level) based on pairwise distance. Each line type denotes the treatment level and suggests an asymptotic trend, which may be offset by increasing the number of samples.

From the 16S rRNA sequencing, 13 major bacteria phyla (**Figure 12, Table 3**) across all the experimental treatment conditions. Some of these phyla included Actinobacteria (40.40 %), DT (17.19 %), Proteobacteria (15.17 %), unclassified bacteria (7.58 %), Acidobacteria (5.10 %), Bacteroidetes (3.56 %), Chloroflexi (3.37 %) and Planctomycetes (3.43 %). Most of the phyla showed significantly different (at $p \leq 0.05$) trends with increasing irradiation (**Figure A6; Table 3**). Pre-treatment, control and low ionizing radiation treatment communities were dominated by Actinobacteria whereas DT dominated higher radiation treatment communities. Overall, with the exception of DT, most phyla showed a consistent trend of decreasing relative abundance with increasing radiation dose (**Figure A6**).

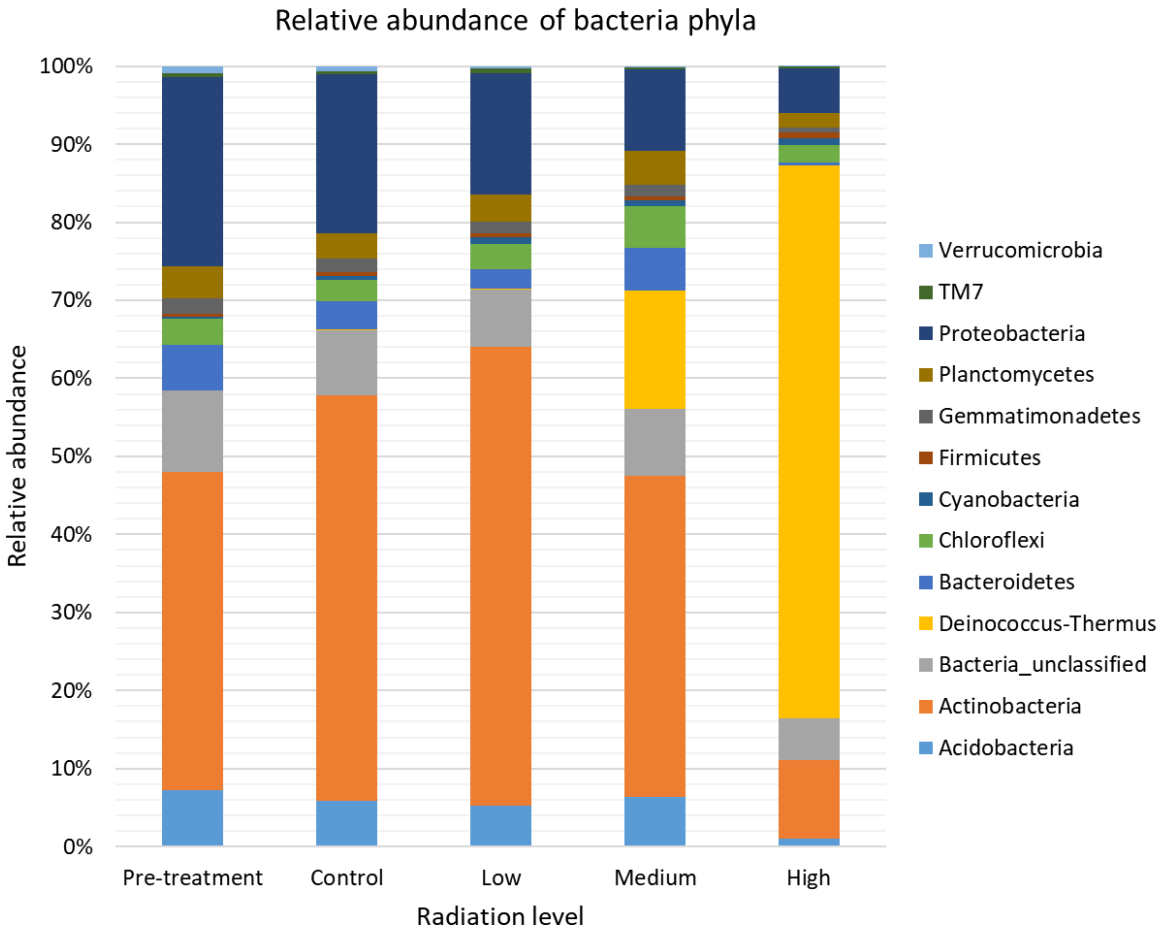


Figure 12. Relative abundance of major bacterial phyla in the study. Dominant phyla differ across the treatment groups. Actinobacteria dominated pre-treatment and control treatments whereas Deinococcus-Thermus dominated the high radiation treatment group.

Table 3. Analysis of variance of the relative abundance of bacterial phyla. The major bacteria phyla with significant P values (at ≤ 0.05) included Actinobacteria, Bacteria unclassified, Chloroflexi, Deinococcus-Thermus, Proteobacteria, TM7 and Verrucomicrobia.

Bacteria phyla	**P-value**	**DF**	**F or X^2 value**
Acidobacteria	0.093	4	7.967
Actinobacteria	**0.000**	4,10	13.600
Bacteria Unclassified	**0.045**	4	9.767
Bacteroidetes	0.103	4,10	2.573
Chloroflexi	**0.025**	4,10	4.497
Cyanobacteria	0.093	4	7.967
Deinococcus-Thermus	**5.08e-10**	4,10	255.500
Firmicutes	0.259	4,10	1.559
Gemmatimonadetes	0.068	4	8.733
Planctomycetes	0.157	4	6.633
Proteobacteria	**0.012**	4	12.900
TM7	**0.022**	4	11.407
Verrucomicrobia	**0.012**	4	12.933

Key: Bold P-values are significant at ≤ 0.05.

To provide a further perspective, we plotted a heat map of the most abundant OTUs, which showed that OTU0001 had the greatest influence on the high radiation treatment community (**Figure 13**). Taxonomic binning of most abundant OTU's suggest that ~80 % of the reads were assigned to *Deinococcus* (**Figure 13**).

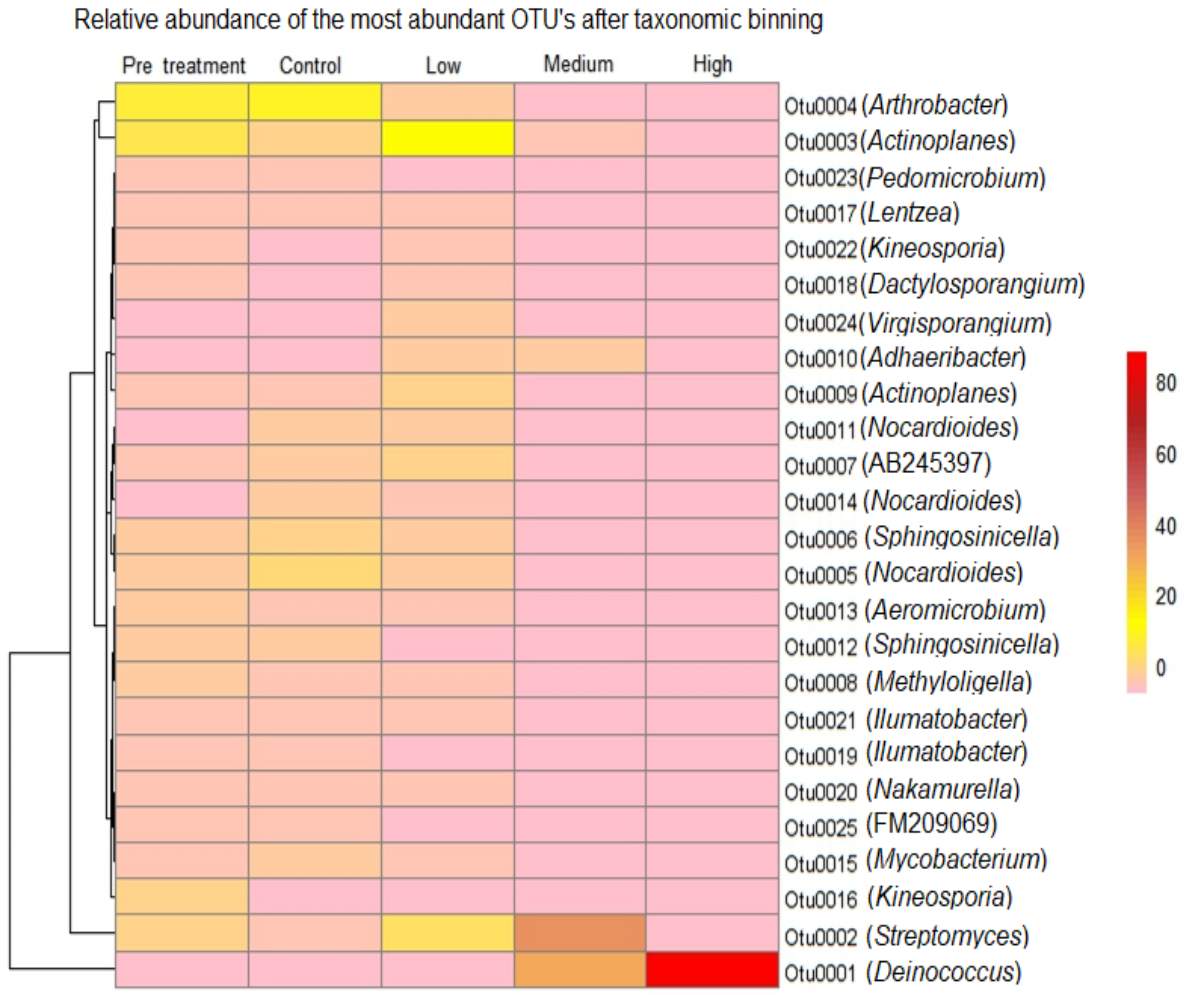


Figure 13. Relative abundance of the most abundant OTUs that are shown to belong to different genera as revealed by taxonomic binning. Independent 'radiation niches' were dominated by different bacterial genera such as *Deinococcus* under high radiation dose and *Streptomyces* in medium radiation dose.

Due to the dominance of *Deinococcus*, a BLAST of all the *Deinococcus* sequences against the NCBI database was conducted and the best-hit scores were obtained (**Figure 14**). Seven known *Deinococcus* species including *D. radiopugnans*, *D. radiodurans* ATCC BAA-816, *D murrayi* DSM 11303, *D. grandis* ATCC43672, *D. geothermalis* DSM11300, *D. ficus* DSM19119 and *D. deserti* VCD115 and one unknown *Deinococcus* (**Figure 14**) were identified from the sequences. Analysis of relative abundances of the *Deinococcus* assemblage showed that they each varied in abundance significantly between treatments (at $P \leq 0.05$) with the exception of *D. radiodurans* and unknown *Deinococcus* OTU (**Table 4**). Preliminary summary of the comparative genome analysis of the different *Deinococcus* species found on NCBI (**Table A4**), which revealed a high guanine-cytosine content in the genomes.

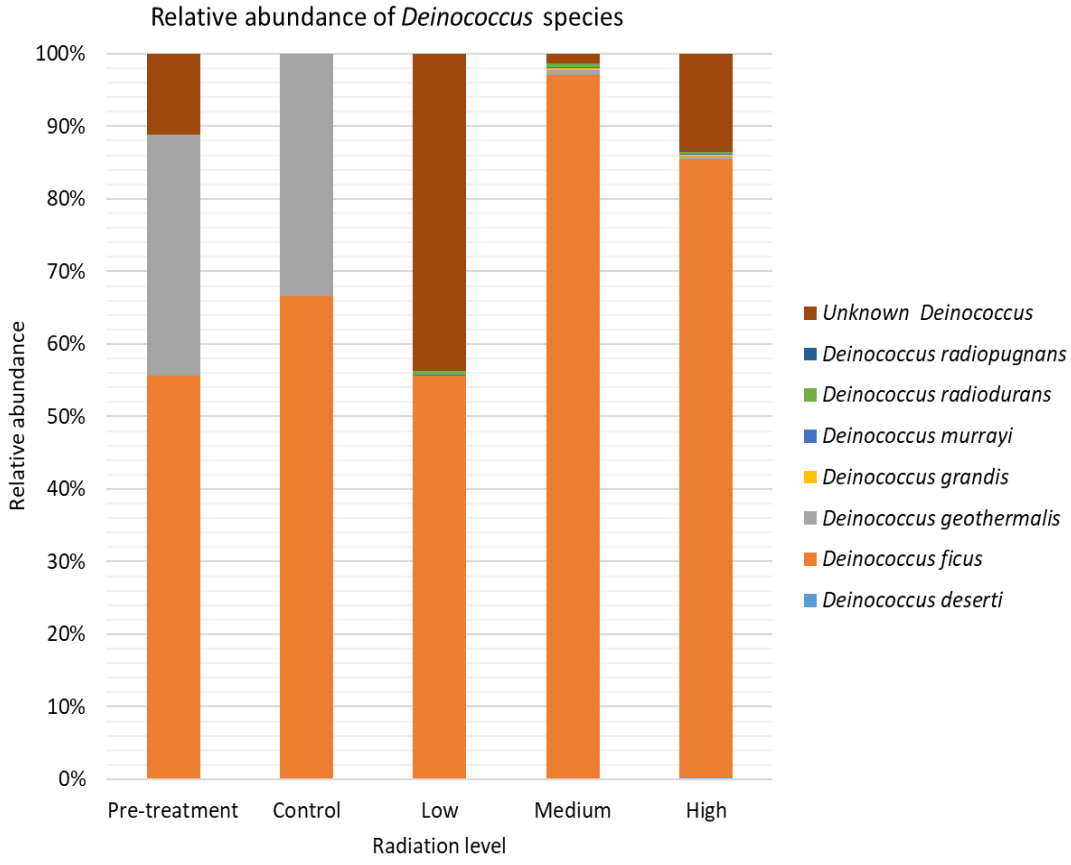


Figure 14. Relative abundance of best-hit scores of the different Deinococcus-Thermus species. Overall, *D. ficus* was the most dominant followed by Unknown *Deinococcus*

Table 4. Analysis of variation of the different *Deinococcus* species obtained in the study. With the exception of *D. radiodurans, D. murrayi* and the unknown *Deinococcus,* the others were statistically significant at $P \leq 0.05$

***Deinococcus* species**	**P value**	**DF**	**F or X^2 value**
D. deserti	**1.59e-08**	4,10	126.80
D. ficus	**6.13e-05**	4,10	21.95
D. geothermalis	**4.32e-06**	4,10	39.38
D. grandis	**0.05**	4,10	3.43
D. murrayi	0.50	4,10	0.91
D. radiodurans	0.08	4,10	2.86
D. radiopugnans	**0.04**	4,10	3.88
Unknown *Deinococcus*	0.09	4,10	2.72

Key: Bold P-values are significant at ≤ 0.05.

To investigate the dominance mechanism of *D. ficus* DSM19119, a comparative genome analysis using clustering of orthologous group (COG) system was undertaken using published genomes of the different *Deinococcus* species. The result suggests that *D. ficus* DSM19119 have higher DNA replication and repairs as well as nucleotide metabolism genes (**Figure 15**). In addition to greater amounts of proteins for cell cycle control, cell division partitioning, cell wall, membranes and envelop biogenesis as well as inorganic ion transport and metabolism (**Figure A7**).

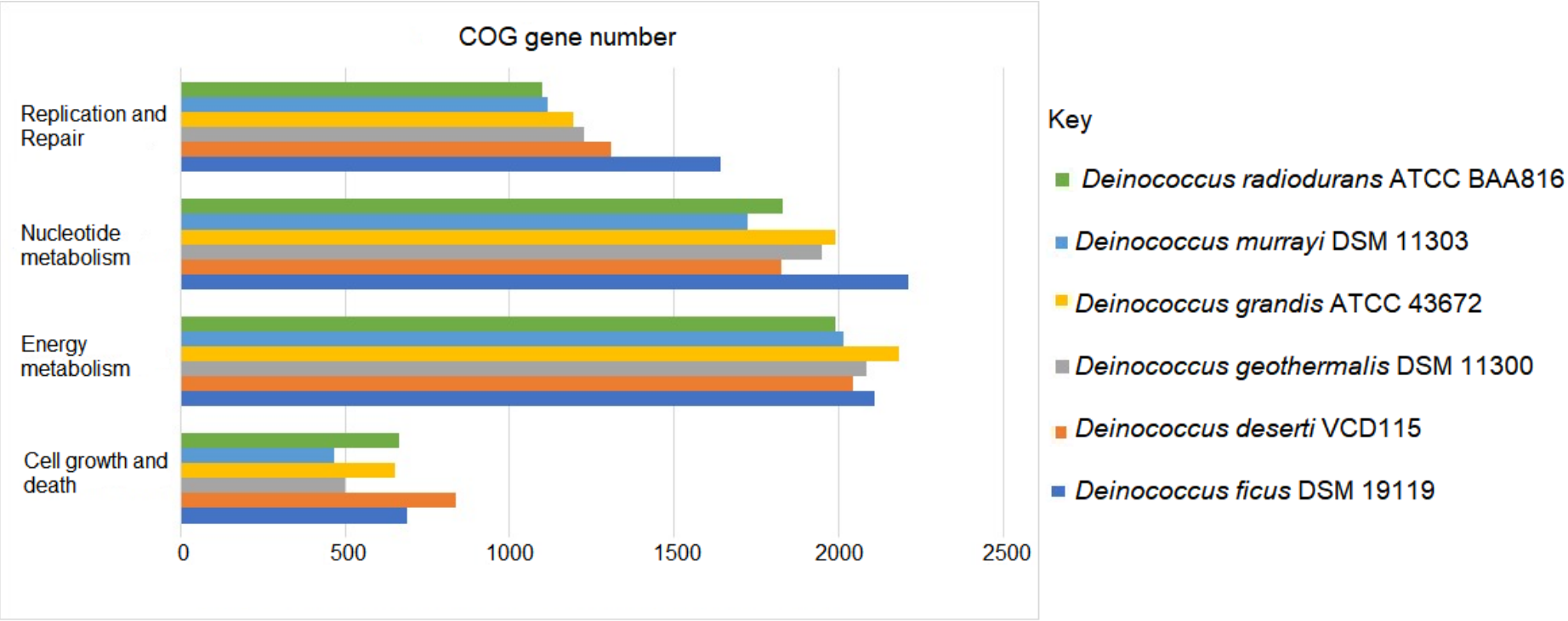


Figure 15. Comparative genome analysis using COG gene copy number of published genomes for the different *Deinococcus* species. *D. ficus* DSM19119 have higher DNA replication and repair as well as nucleotide metabolism, which may account for their dominance in the study.

RAST subsystem comparison for these abundant *Deinococcus* genomes also reveals higher amounts of cofactors, vitamins, prosthetic groups and pigments, membrane transport, protein metabolism, fatty acids, lipids and isoprenoids, nitrogen metabolism, and amino acids and derivatives related proteins in *D. ficus* DSM19119 compared to the other genomes (**Table A5**). However, *D. geothermalis* DSM11300 and *D murrayi* DSM 11303 have more CRISPR proteins compared to *D. ficus* DSM19119 (**Table A6**). Different *Hymenbacter* species were abundant in the low and medium radiation treatment communities (**Table A7**) and have been previously suggested to tolerate irradiation (Lee et al., 2017; Srinivasan et al., 2017).

Functional gene analysis of DT and the whole bacterial community suggest an abundance of amino acid and derivatives, clustering based subsystems, protein metabolism, and carbohydrate-related genes under high radiation conditions (**Figures 16** and **17**). Furthermore, the annotation of SEED subsystems level 3 DNA metabolism-related genes for Deinococcus-Thermus revealed higher DNA replication under high irradiation condition (**Figure A8**). However, the functional diversity of these genes increased with increasing radiation dose for DT (**Figure 18**) but decreased for the whole bacterial community especially under high radiation (**Figure 19**). The possibility of these effects being attributed solely to legacy effects from legacy DNA can be discounted with confidence as the read lengths were the same for the different treatments considered in the study

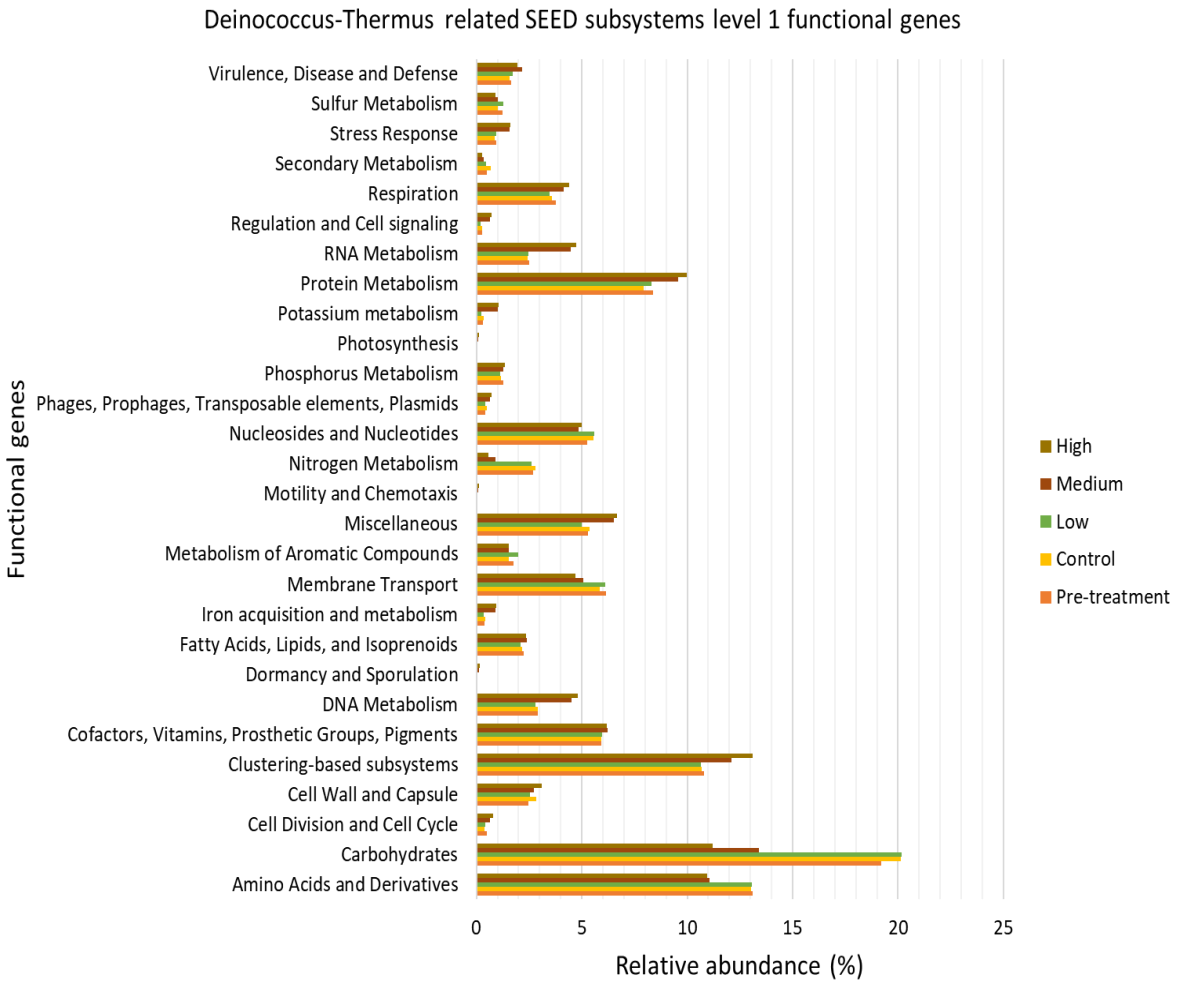


Figure 16. Abundance of SEED subsystems functional genes in Deinococcus-Thermus annotated from sequences available on MG-RAST. There was an abundance of amino acid and derivatives, clustering based subsystems, protein metabolism, and carbohydrate-related genes under high radiation conditions.

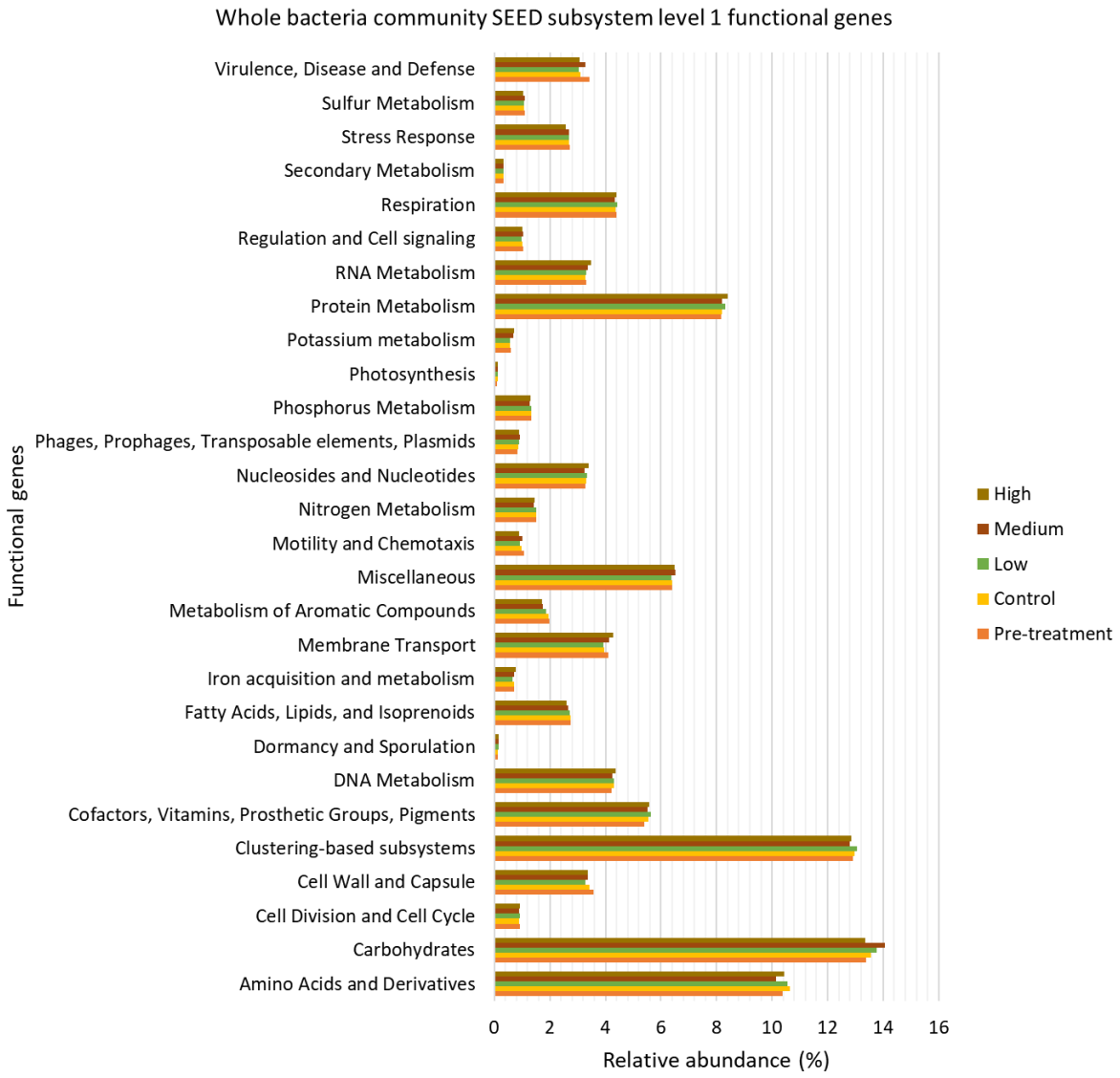


Figure 17. Abundance of functional genes in the bacterial community annotated from sequences available on MG-RAST. There was an abundance of amino acid and derivatives, clustering based subsystems, protein metabolism, and carbohydrate-related genes under high radiation conditions.

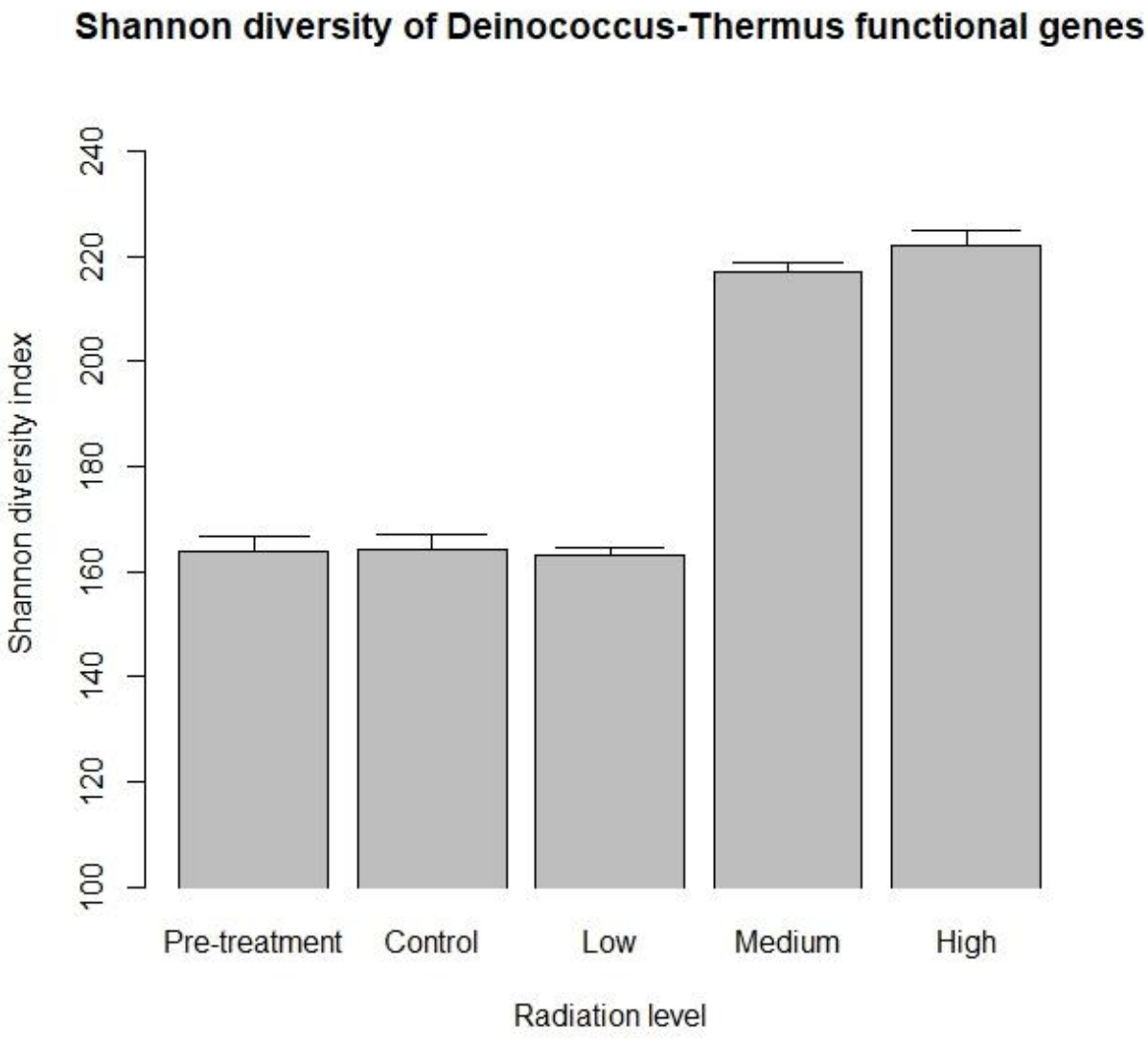


Figure 18. Functional gene diversity of Deinococcus-Thermus annotated from available sequences on MG-RAST. Functional gene diversity of the phylum increased with increasing radiation dose.

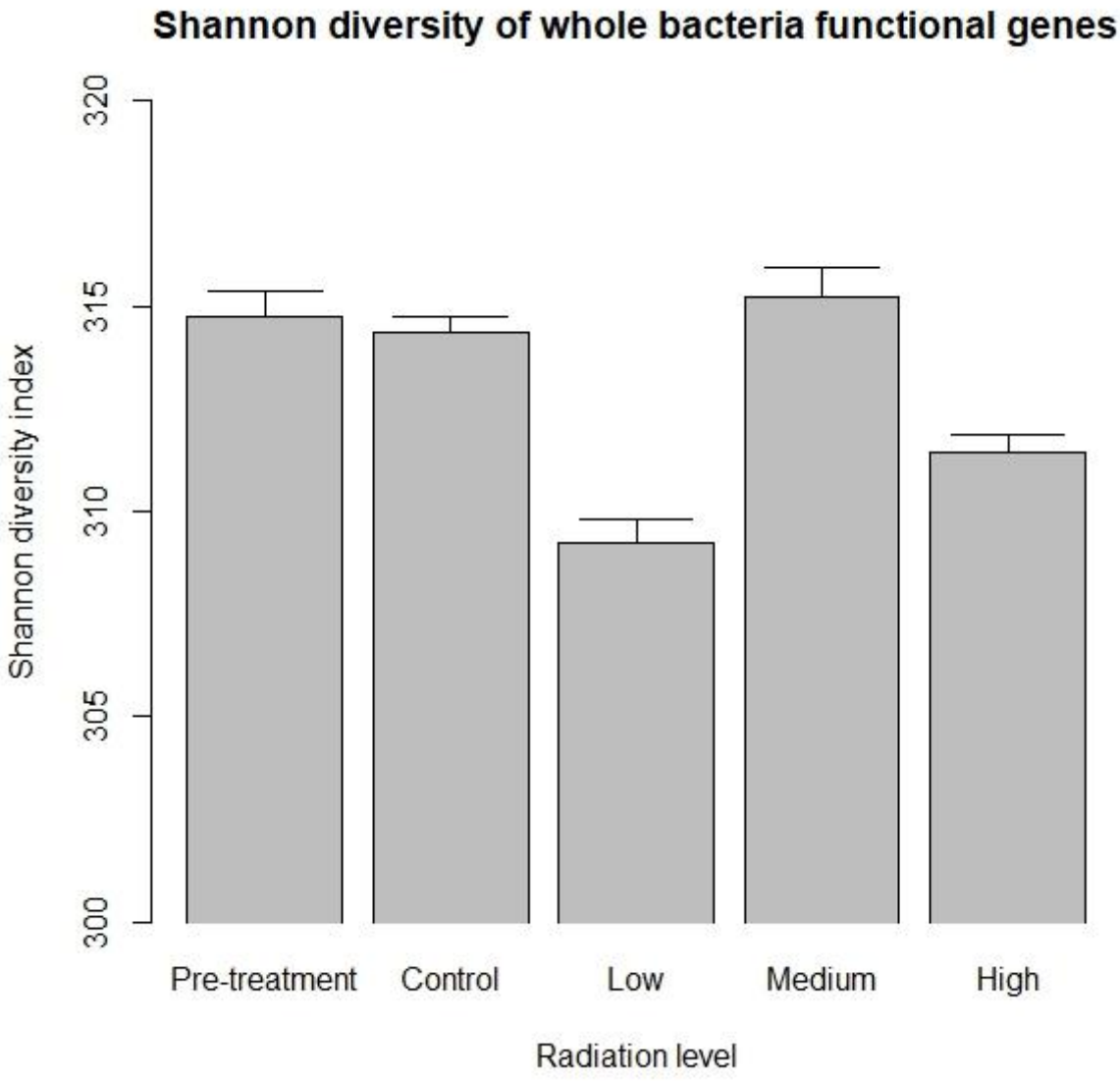


Figure 19. Functional gene diversity of the whole bacterial community annotated from available sequences on MG-RAST. Functional gene diversity of the bacterial community decreased under high radiation treatment.

Diversity indices were applied to characterize and compare the soil communities (Lemos et al., 2011) resulting from the treatments. Four alpha diversity measures were applied to analyse the species diversity within each soil bacterial community including ACE, Chao, Shannon, and Inverse Simpson indices. A consistent trend of decreasing diversity with increasing radiation exposure was observed in OTU alpha and phylogenetic diversity of the community (**Figure 20, Table 5**). In the same way, the diversity of *Deinococcus* also decreased with increasing radiation exposure, which is similar to those of the overall community assemblage (**Figure 21**). Analysis of phylogenetic diversity was used to measure differences in bacterial community complexity among the treatment conditions (**Figure A9**). Phylogenetic diversity decreased with increasing radiation intensity. Abundance models including broken-stick, geometric, and log series (Smart, 1976; Newman and Clements, 2007) suggest that the bacterial community abundance within each treatment increased significantly with increasing ionizing radiation intensity (**Figure A10, Table 5**). The goodness-of-fit of the communities to these models suggest increasing competition due to ionizing radiation. However, OTU community richness decreased significantly with increasing ionizing radiation (**Figure 22, Table 5**). The OTU richness of *Deinococcus* had richness pattern distinct from those of the whole community by increasing in spite of the high radiation conditions (**Figure 22**).

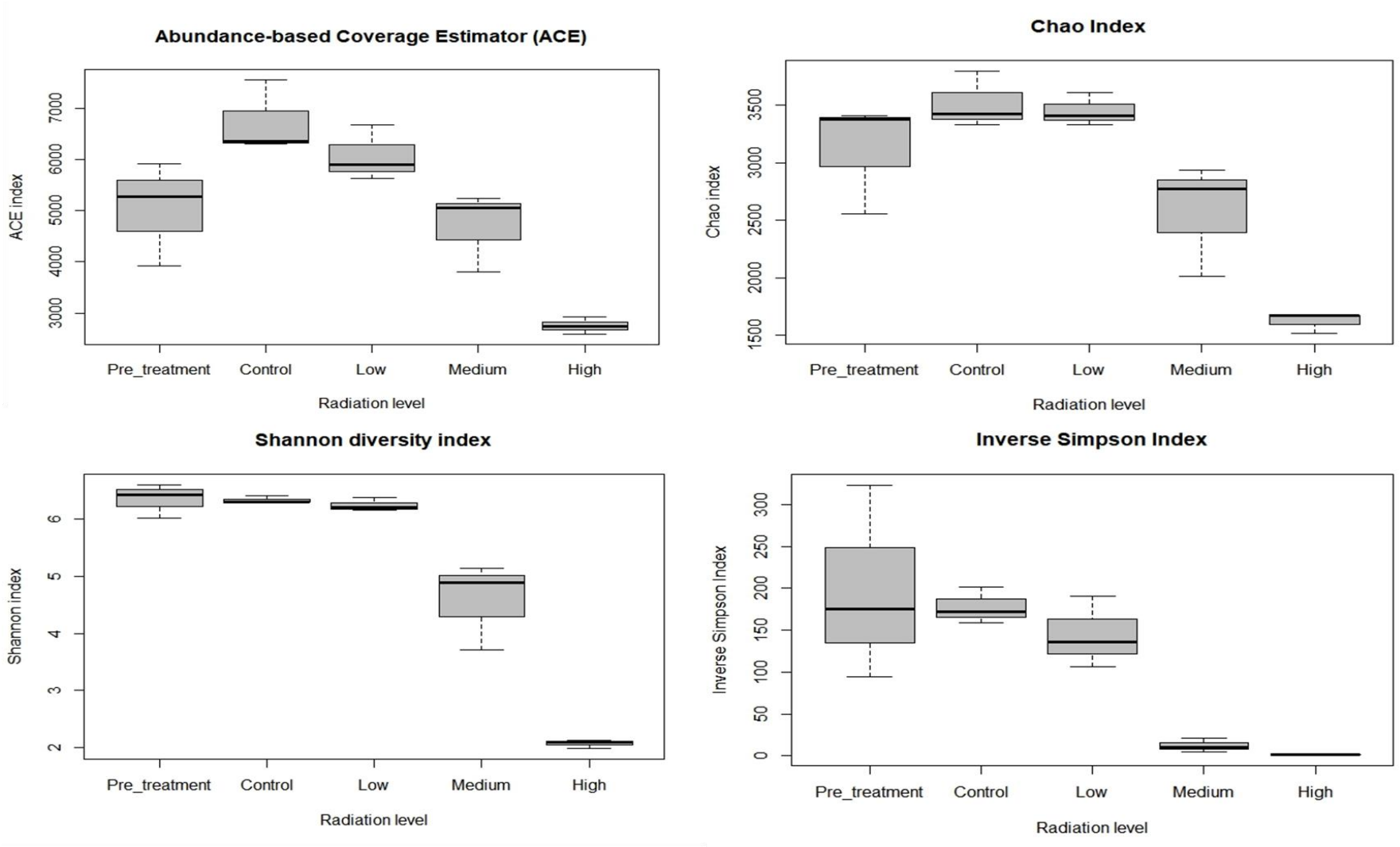


Figure 20. Alpha diversity indices for the different treatment conditions in the study. Diversity decreased proportionally with increasing radiation treatment.

Table 5. Analysis of variance for the different abundance models, richness and diversity indices. OTU abundance, richness, alpha, beta and phylogenetic diversity were significantly different.

Abundance models	P value	DF	F or X^2 value
Broken stick	**4.34e-07**	4,10	64.09
Geometric series	**3.82e-07**	4,10	65.81
Log series	**1.12e-06**	4,10	52.49

Diversity and richness	P value	DF	F or X^2 value
OTU richness	**1.78e-06**	4,10	47.600
ACE	**0.000**	4,10	14.110
Chao index	**0.000**	4,10	16.420
Shannon index	**2e-07**	4,10	75.330
Inverse Simpson index	**0.003**	4,10	8.200
Faith's phylogenetic diversity	**0.018**	4	11.867
True beta diversity	**0.050**	4	9.067

Key: Significant P value in bold

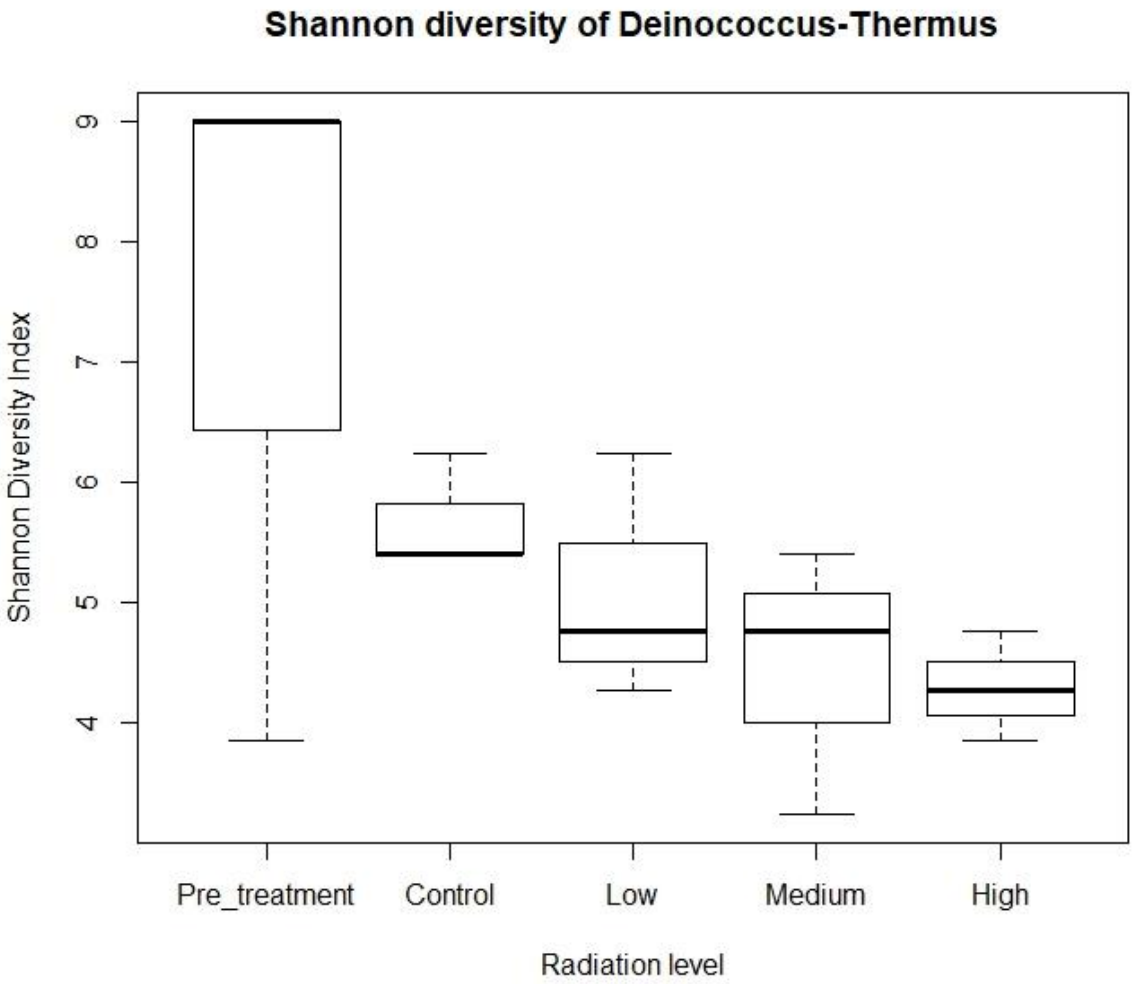


Figure 21. Species diversity index of Deinococcus-Thermus. Species diversity decreases as radiation intensity increases, which is similar to the overall community pattern.

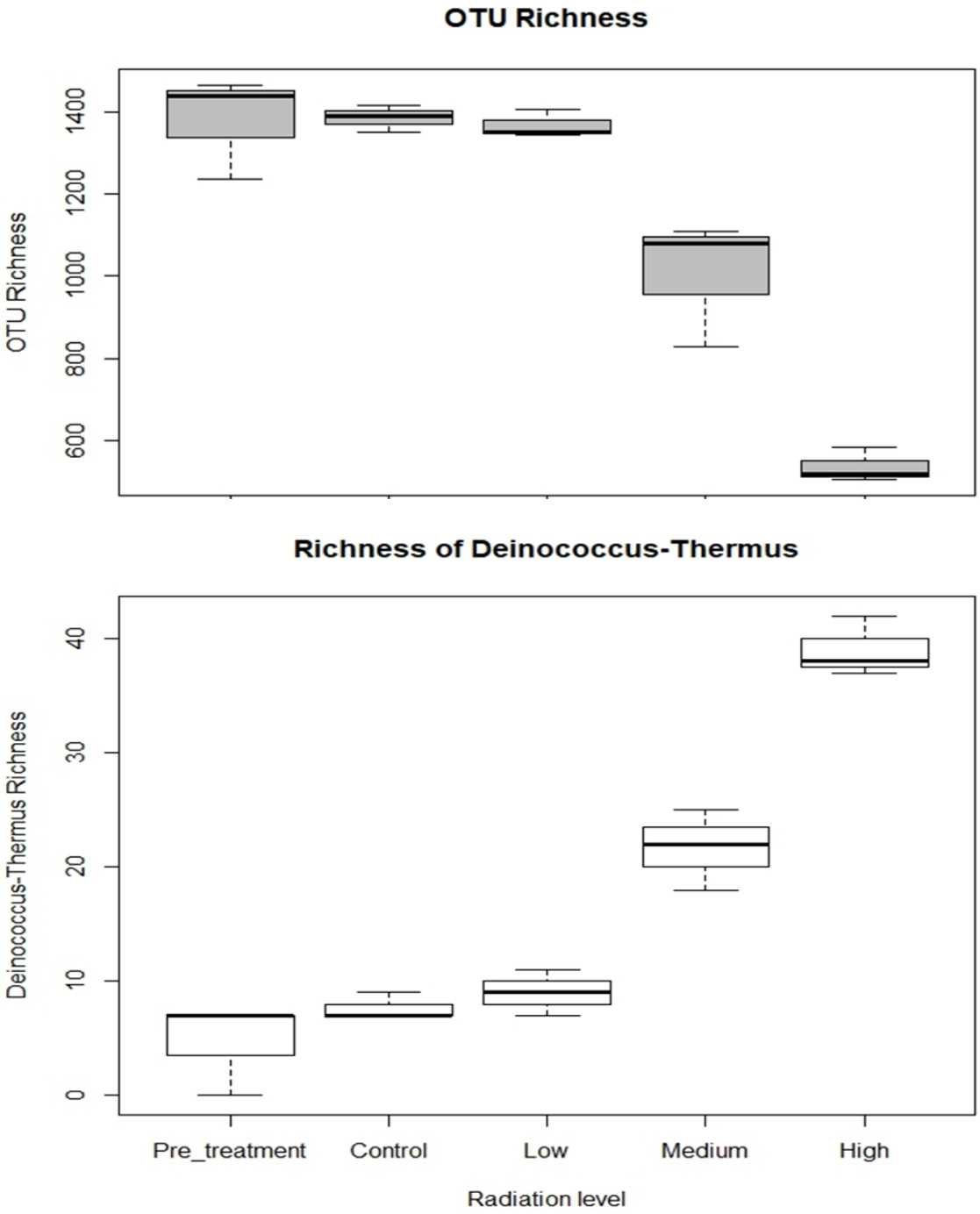


Figure 22. OTU richness of whole bacterial community and Deinococcus-Thermus. OTU richness (of all phyla) decreased significantly with increase in ionizing radiation treatment (at a coverage of >97 %) while the species richness of Deinococcus-Thermus increased with increasing radiation intensity.

To observe the impact of the taxonomic trend in the community, a non-metric multidimensional scaling (NMDS) and Bray-Curtis dissimilarity analysis were conducted (**Figure 23**). The NMDS analysis of OTU revealed a trend consistent with the taxonomic composition of each treatment condition. Three main clusters comprising of pre-treatment, control and low radiation dose formed one cluster, while medium and high radiation treatment make up the other two clusters. The clustering together of pre-treatment, control and low radiation may suggest similar community composition. However, to ascertain if each treatment community forms a distinct from others, the OTU abundance data was subjected to dissimilarity measure based on Bray-Curtis. The results showed that each treatment community is more similar within than between each other (**Figure 23**) i.e. they are distinct. Two cluster groups were formed similarly to those formed from NMDS ordination. The results of the Bray-Curtis analysis emphasized the patterns observed from NMDS ordination of the communities.

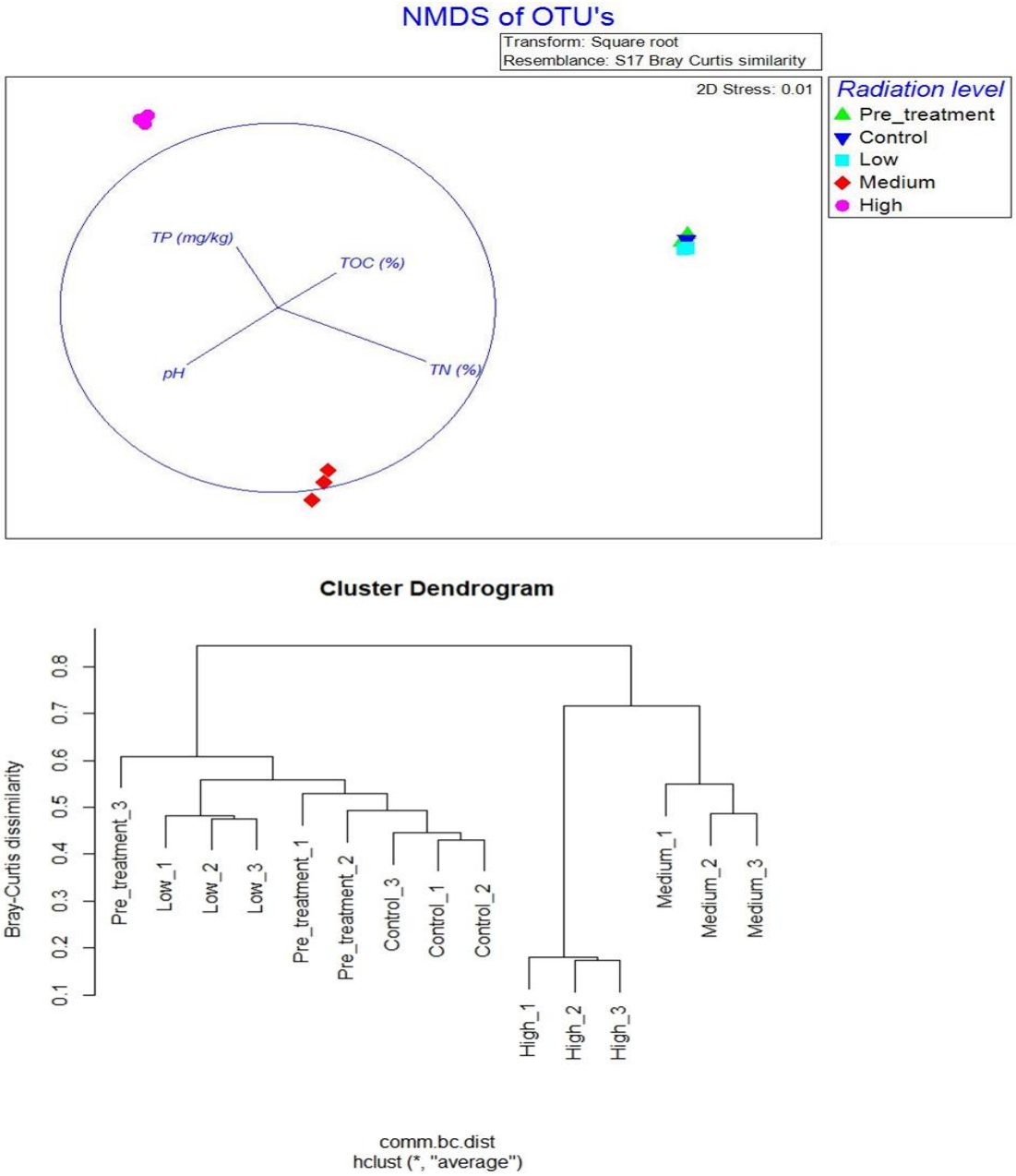


Figure 23. Non-metric multidimensional scaling (NMDS) and Bray-Curtis dissimilarity analysis used to visualize the multivariate structure of the bacterial communities in the study. The NMDS analysis suggests that distinct bacterial communities resulted from the high and medium treatment conditions whereas pre-treatment, control and low radiation treated communities were almost similar. Dissimilarity measure based on Bray-Curtis led to the formation of two clusters composed of pre-treatment, control and low radiation dose samples while medium and high radiation treated groups make up the second cluster.

3.4. Discussion

From the 16S rRNA data, as anticipated *Deinococcus* emerged as the most abundant genus under medium (1 kGy/hr/wk) and high (3 kGy/hr/wk) radiation conditions. This section is broken down in terms of the major questions posed by this study:

1) How does the community structure of Deinococcus change with increasing radiation exposure, and do different Deinococcus forms dominate at different radiation intensities – suggesting the existence of 'radiation niches'?

Compared to the pre-treatment, control and low radiation treatments, the relative abundance of *Deinococcus* in the soil bacterial community showed a major increase in the medium and especially the high radiation exposure treatments. In the highest radiation treatment, more than 80 % of 16S reads in the amplicon dataset were attributed to *Deinococcus.* Several previously described *Deinococcus* species were found in the samples, as well as one undescribed *Deinococcus* OTU (**Figure 14; Table 4**). All of the described *Deinococcus* species increased in relative abundance with increasing radiation exposure (**Figure 14**), but the proportionate increase in abundance differed between species, with *D. ficus* overwhelmingly dominant in the high radiation treatment. The difference in relative abundances of the various *Deinococcus* species increased with exposure levels, supporting the existence of different preadapted 'radiation niches'. *D. ficus* alongside *D. geothermalis* dominated the pre-treatment and control niches, low radiation niche was dominated by *D. ficus* and the unknown *Deinococcus* while *D. ficus* clearly dominate higher radiation niches (**Figure 14**). These differences presumably also relate to the tolerance of UV light or drying that is considered as a major selective factor behind the combined characteristics that render *Deinococcus* resistant to radiation (Pogoda et al., 2005). Despite the increase in relative abundance, *Deinococcus*

diversity decreased with increasing radiation dose. There appears to be a high degree of determinism in the development of the *Deinococcus* communities following radiation exposure, as each replicate of the same treatment had the same group of *Deinococcus* species at similar relative abundances, clustering very closely together on an ordination.

Soil bacterial diversity in general also decreased with increasing radiation exposure (**Figure 20, Table 5**). Our results concerning diversity disagree with those of Ragon et al. (2011) which suggested that bacterial diversity in highly irradiated samples is comparable to that in less irradiated sites. The differing observations in both studies may be attributed to soil type, the target region for sequencing, diversity measures adopted and concentration of irradiation. However, the diversity pattern reported in this study is similar to that reported after radiation treatment of desert soil in Rainey et al. (2005) where the diversity of bacteria decreased as the dose of γ radiation increased. They also reported the dominance of *Deinococcus* in the community alongside *Geodermatophilus*, and *Hymenobacter*. However, in this study, there was no increase in the former genera but the latter increased exponentially under the low and medium doses (**Table A7**; see discussion on hypothesis 3 below). In Rainey et al. (2005), the number of *Deinococcus* species recovered by culturing from the arid soil (i.e. 60) is much higher than the eight obtained from blasting our sequences with the NCBI database (**Figure 14; Table 4**).

Additionally, the results obtained in this study corroborate previous findings by Romanovshava et al (1998) from a 10-km zone around the Chernobyl Nuclear Power Plant, which showed the persistence of broad bacterial taxa in radiation-exposed soils despite a reduction in diversity resulting in the formation of a distinct taxonomic assemblage. In this study, the most striking change in community composition was the dominance of *Deinococcus* under high radiation exposures. This is in line with the report of Ryan et al. (2008) that

irradiation selectively eliminates radiation-sensitive bacteria while encouraging the persistence of radiation-resistant bacteria.

The changes in the relative abundance and diversity of soil bacteria observed in the present study can be attributed to the extreme conditions imposed by the ionizing radiation treatments, which likely influenced basic cellular functions. These changes caused a decrease in the relative abundance and diversity of most phyla (**Figures 12** and **20**) and an increase in *Deinococcus* abundance and functional gene diversity under irradiation (**Figures 13** and **18**). In a related study, Liu et al. (2017) suggested that the slow-growing nature and absence of a resting phase in more radiation-sensitive bacteria would increase the impact of ionizing γ radiation on them. Members of DT are amongst the most extremophilic bacteria known (Theodorakopoulos et al., 2013). In the most extreme natural conditions including non-irradiated arid environments, *Deinococcus* are often implicated in constituting the main differences in bacterial communities (Pereira et al., 2014; Li et al., 2015a) as recorded here.

Among the bacterial taxa that were reported by El-Sayed and Ghanem (2009) as being significantly resistant to irradiation, only Actinobacteria and Chloroflexi were found to be relatively resistant in our study and this may partly be due to differences in soil type between the studies. This is supported by the findings of McLaren (1969), who suggested that the effects of gamma irradiation depend on soil type and the background microbial communities. Moreover, in El-Sayed and Ghanem (2009), all the γ irradiation was carried out in one burst without giving the community time to adjust resulting in a greater selective impact on the microbial community structure. El-Sayed and Ghanem also reported that bacterial diversity remained unchanged despite their radiation treatments. Their finding is not supported by the results from our study, which showed that as radiation dose increases, community and species diversity of radiation resistant taxa (DT) decreases.

2) What features of the genomes of the more abundant species of Deinococcus in the community might confer greater 'success' at high radiation exposures, compared to other less abundant species?

The physiological basis of radiation resistance in bacteria and eukaryotes is apparently a pre-adaptation linked to desiccation resistance (Musilova et al., 2015; Beblo-Vranesevic et al., 2018) involving the combination of other mechanisms with DNA repair capabilities to prevent cellular damage (**Figures 12, 13, 15,** and **16**; Mattimore and Battista, 1996; Cox and Battista, 2005, Rainey et al., 2008). The functional annotation of the whole bacteria community and Deinococcus-Thermus (**Figures 16** and **17**) reveal the same set of genes but different abundance. This is in support of the findings of (Makarova et al. 2001; 2007) which suggest that radiation-resistant *Deinococcus* species produce the same kind of proteins. However, our results disagree with the aspects of their results that implies that certain proteins (like DNA-repairs) are produced in similar amounts as we found higher amounts of DNA metabolism proteins (**Figures 16** and **A8**) in *Deinococcus*, which may correlate with their greater success in the community.

A more specific mechanistic explanation for the radiation resistance of *Deinococcus* was presented by Ngo et al. (2013) involving the RecA (dependent and independent) proteins, which has been implicated in genome reconstitution after exposure to extreme levels of ionizing radiation by exhibiting active and inactive states that are readily interconverted in response to several sets of stimuli and conditions. These proteins ensure that the genetic integrity of the DNA is restored before the cycle of cell division (Cox and Battista, 2005). The S-layer (proteinaceous cover) is also thought to be important in protecting the protein structures of radio-resistant bacteria species such as *Deinococcus* species (Ghedira et al., 2016). This S-layer is important in the application of these resistant microorganisms to control the different processes involved in the stability of radioactive waste inventories in nuclear storage and

disposal facilities. In addition, high intercellular manganese/iron concentration ratios have been implicated to enhance *Deinococcus* radiation resistance ability by preventing the accumulation of iron-dependent reactive oxygen species and enhance enzymatic processes that are required to repair their DNA for survival (Fredrickson et al., 2008, Daly, 2009). Proteins are the most important molecules under irradiation conditions as Daly (2009) suggested that the extent of protein damage under irradiation is more related to survival than DNA damage. Some of these resistant species have lower protein oxidation levels compared to sensitive bacteria but have similar frequencies of DNA double-strand breaks (Slade and Radman, 2011).

As identified by White et al. (1999); Hess (2003), the chromosomes and mega plasmid of DT taxa contain multiple genes for DNA repair, DNA damage export, desiccation, starvation recovery and genetic redundancy occurring together, a package of genes distinct from those required to survive in a normal mesic soil environment. DNA repairs and replication-related genes were found in the study with the latter been greatest under high irradiation conditions (**Figure A8**). Earlier, Redon and Bonner (2011) have suggested that under extreme conditions, efficiency in DNA replication is required for proliferation. The abundance of this DNA metabolism-related gene in high irradiation niches may also account for the abundance of Deinococcus species while suggesting their efficient utilization. In Slade et al. (2009), whilst enumerating the replication systems in *D. radiodurans*, they implicated the abundance of repairs and replication-related proteins to their radiation resistance abilities. Among these proteins is *recA*, which is core to genomic restoration in most *Deinococcus* after acute irradiation (Liu et al., 2003).

Comparison of the published genomes of the same *Deinococcus* species found in our microcosms suggests that the dominant species *D. ficus*, and other abundant species of *Deinococcus* have functional differences that may help explain their distinct abundance under high radiation conditions. In terms of the differences in genome structure that might explain its

predominance, the genome of *D. ficus* possesses a greater number and proportion of genes related to DNA repair and nucleotide metabolism, cell wall, membrane and envelop biogenesis as well as cell cycle control, cell division and chromosome partitioning related genes in addition to a higher guanine-cytosine ratio, compared to other *Deinococcus* species recorded in the study (**Figures 15, 18** and **Table A4**). Of particular interest is that *D. geothermaliis* and *D. murrayi* contain the greatest number of CRISPR-associated proteins (**Table A6**), but Makarova et al. (2007) suggest these proteins have no role in determining the levels of resistance in *Deinococcus* species. In addition, *D. ficus* is capable of utilizing diverse carbon sources derived both from microbial cells killed by the radiation and plant-derived organic matter in the soil. This includes several C5-C12 containing compounds like L-arabinose, lactose, D-trehalose, D-xylose, D-mannose, D-melibiose, N-acetyl-D-glucosamine, D-sorbitol, lignin, cellulose and hemicellulose (Lai et al., 2006). Moreover, in this study, *D. ficus* had the greatest amount of carbohydrate enzymes (**Table S4**). The carbon derived from the diverse sources supports functional processes as well as the accumulation of nucleotides (Ghosal et al., 2005). *Deinococcus* species have higher catalase and superoxide dismutase activities due to manganese accumulation throughout their cells as well as carbon oxidation processes, which increases their sensitivity to the DNA-damaging agents by regulating the functions of iron in dividing cells (Chou and Tan, 1990; Daly, 2009; Slade and Radman, 2011). On the hand, their pH range, fatty acid compositions, and other biochemical parameters, such as the ability to grow in minimal medium without yeast extract (Ferreira et al., 1997) are some of the features that can be used to distinguish the different radiation-resistant *Deinococcus* species. Together, these passive and active biochemical adaptations enable *Deinococcus* cells to compensate for the detrimental protein and DNA damage induced by ionizing radiation (Cox and Battista, 2005).

A possible explanation for the dominance of D. *ficus* in our study is that the colonization of the high radiation mesocosm niche by D. *ficus* involves greater access to limiting resources, allowing faster growth to outcompete radiation intolerant taxa (including other *Deinococcus*) within the soil microcosm (Spiers, 2014). *D. ficus* was already detectable as a member of the pre-treatment and control samples but greatly increased in abundance as radiation intensity increased. The greater amount of transmembrane proteins in *D. ficus* (**Table S4**) may have also contributed to their overall dominance of high irradiation niches, since these proteins have been previously suggested to play complementary roles to the DNA repair machinery in *Deinococcus* (Hassan and Gupta, 2018).

3) Are there other groups of bacteria, which increase in relative abundance in response to radiation?

Amongst the other groups of bacteria present in the soil mesocosms, the assemblage of phyla and their relative abundance was distinct between the high radiation and other treatment conditions. Aside from *Deinococcus* some other bacteria groups showed an increase in relative abundance under radiation treatment, at least in the medium and low radiation exposure treatments. In this treatment, the second most abundant OTU was an unclassified species of *Streptomyces* (**Figure 13**). This genus is known to contain various radiation resistant species (Mao et al., 2007; Mohammadipanah and Wink, 2016) including species that are tolerant to acute doses of gamma irradiation (Bhave et al., 2013), although they have never been studied in a community perspective before. Nonetheless, the radiation-tolerance of certain known *Streptomyces* may be attributable to their genome plasticity-induced mutagenic DNA break repairs (Stonesifer and Baltz, 1985; Hoff et al., 2018), which is quite similar to the RecA mechanism involved in the ability of *Deinococcus* to resist high radiation doses.

In addition to *Streptomyces*, *Hymenobacter* species were abundant in the low and medium treatment mesocosms including *H. actinosclerus, H. aerophilus, H. antarticus, H. fastidiosus, H. ocellatus, H. roseosalivarius, H.* sp. VUG-A141A (**Table A7**). Collins et al. (2000), Lee et al. (2014) and Lee et al. (2017) have previously reported the radiation-resistance of this genus. Lee et al. (2017); Srinivasan et al. (2017) suggested that the gamma radiation resistance mechanism of *Hymenobacter* may be related to genomic guanine-cytosine content, enzymes for nucleotide excision repairs, DNA hybridization potential and the ability to accumulate manganese to prevent harmful effects from ROS. These mechanisms are similar to those used by *Deinococcus* species to resist ionizing radiation damage.

4) What does the profile of functional genes present in the total soil metagenome – and in the known published genomes of the dominant bacteria present – indicate about the functional capabilities and resilience of the irradiated soils?

In this study, functional genes, as predicted from previously uploaded sequences on MG-RAST, decreased with respect to the whole community but increased for DT (**Figures 18** and **19**). There was an increased abundance of clustering-based subsystems, carbohydrate and amino acids related genes in combination with other genes. The survival of bacteria in a radiation-contaminated soil is likely due to the ability of their functioning mechanisms to efficiently neutralize peroxide compounds and repair radiation-damaged DNA, through nucleotide excision repair pathways (Romanovskava et al., 1998; Kisker et al., 2013). Specifically, the role of intracellular Mn(II)/Fe ratio in DNA repairs and protection of protection of proteins from oxidative damage (Fredrickson et al., 2008) in *Deinococcus* species is essential to the patterns observed in the community

The non-mobilization of phosphorus may be attributed to the lack of phosphorus solubilizing activity, which normally depends on a range of Proteobacteria species. A group

(i.e. Proteobacteria) that decreased increasingly with higher irradiation doses (**Figure 12**). Previously, Makarova et al. (2007) reported that the accumulation of high intracellular Mn (II) concentrations by Mn-transporter *nramp*, and proteins DdrF, DdrJ and DdrK, were necessary for radiation resistance in *Deinococcus geothermalis* and *D. murrayi* alongside a radiation response regulon, palindromic DNA motif. Although some radiation resistance genes present in those species are absent in *D. ficus* and *D. deserti* (like CRISPR associated proteins (**Table A6**)) - they contain enzymes involved in nitrogen metabolism, including nitrate and nitrite reductases (Matrosova et al., 2017). Together, these suggest they may be utilizing a different but related mechanism for radiation resistance. Ionizing radiation such as gamma rays causing cellular damage can activate reactive oxygen species (ROS) response genes and related systems, to initiate ROS-scavenging that protects cells against oxidative damage caused by desiccation (Li et al., 2015b; Jeon et al., 2016). *Deinococcus* species have been reported (Patel et al. 2009) to withstand desiccation, and effects of ROS. In addition, under irradiation, chemotropism increased through cellulolytic, nitrifying, and sulfate-reducing processes in the community bacteria in contaminated soil was found to be 1-2 orders of magnitude less than in control soil, indicating the strength of the effect of anthropogenic radiation on the abundance and diversity of soil bacteria (Romanovskava et al., 1998).

3.5. Concluding Remarks

In conclusion, this study has highlighted some of the complexities in the community ecology of irradiated soils. Multiple *Deinococcus* species increase in abundance in the irradiated soil community, but their community composition varies with radiation level. The dominant species at high radiation, *D. ficus*, possess various features, which may explain its stronger radiation resistance, or stronger competitive ability under high radiation conditions compared to other *Deinococcus*. There were other non-*Deinococcus* radiation-resistant species detected, but all of these peaked in the 'low' and 'medium' radiation niche, and decline in abundance at high radiation intensity. It would be interesting to study the soil functional implications of the shift in community composition towards *D. ficus* in a soil, and how this might impact ion mobility etc., traits of interest in terms of radioactive contamination of soils.

It is unclear how other soil types would differ in terms of their bacterial community under irradiation, and in particular their *Deinococcus* community. For example, soil pH, nutrient concentration, oxygen concentration and level of hydration might all affect which *Deinococcus* species - if any - becomes dominant. Transcriptome-based studies would more clearly distinguish 'legacy' DNA from dead cells from that in currently living cells. It would also be interesting to use continuous radiation doses, over longer periods, while measuring soil functions such as respiration and N fixation.

PART 4. CONCLUSION

4.1. Changes in Functional Genes and Taxonomic Composition due to ^{60}Co Gamma Irradiation

The experimental framework adopted in this study enabled the assessment of the functional gene and taxonomic composition of a soil community subjected to gamma irradiation. As anticipated, there was a greater abundance of prokaryote than eukaryote taxa in the post-radiation community. This is likely due to the structural and composite nature of prokaryotic cells, which are simpler than the complex cells of eukaryotic organisms. Eukaryotic cells are more susceptible to oxidative stress and genetic damage than prokaryotic cells. The acute ionizing radiation caused a greater excitation of eukaryotic cellular molecules and the release of free radicals and ROS (Hurem et al., 2017), which contributed to the reduced diversity observed in the studies. However, this led to an increase in functional genes at especially under high irradiation doses. Therefore, it can be concluded that there is a greater functional burden from irradiation as edaphic microorganisms attempt to offset the stress impacts of the irradiation. Moreover, a cascade of functional gene systems is required to outlive ionizing radiation burst including the activation of possibly hitherto inactive genes. However, these effects do not appear to operate independently from the environmental conditions and soil type. Thus, it may be concluded that aside from the dose and time-dependent taxonomic and functional response by soil microbial communities to irradiation, the soil type and prevailing environmental conditions contributed to the observed patterns reported here.

Due to their abundance in the post-radiation community, some eukaryotic and prokaryotic phyla were suggested to contain radio-tolerant taxa and these require further investigation to understand the functional mechanisms involved. The study showed the capacity of soil microbial community to remain viable under the different doses of ionizing gamma irradiation considered. This consideration is vital in view of the widespread application of gamma radiation to sterilize soil and other important materials.

4.2. Dominance of Radiation-Resistant Taxa in Soil Bacteria Community after ^{60}Co Gamma Irradiation

The composition of the soil bacterial community shifted under the effect of gamma irradiation to allow the dominance of radiation-resistant *Deinococcus* species and others like *Hymenobacter* and *Streptomyces*. These dominant species established radiation-niches along the different radiation doses considered in support of dose-dependent interactions. Clustering-subsystems, carbohydrate and amino acids and derivatives response genes were vital in sustaining these interactions. In addition, an array of gene regulated processes and functions alongside the standard DNA repair mechanisms required by radiation resistant species. In possible future application, it would be valuable to combine the genomes of these species to create a model organism that can be used to bioremediated soil contaminated by gamma irradiation. Genes from these mutant organisms can also be transferred to other organisms with a view to transferring the potential to resist acute doses of gamma irradiation.

The 16S rRNA approach improved upon our general understanding of bacterial community responses to environmental change induced by an ionizing radionuclide. For instance, the finding that *Deinococcus ficus* can be used as an indicator of high gamma irradiation conditions and the potential physiological conditions of the resulting soil systems. The reduced abundance of bacteria species supports the conclusion that viable microbial life and metabolic activity can persist after a radiation-induced disturbance. Furthermore, the functional prediction from the community highlighted key physiological processes that result from irradiation that in turn enabled hitherto non-abundant radio-resistant taxa to dominate the community. It is possible to conclude that the radiation dose and rate combined with the environmental conditions selected the abundant taxa based on their functional traits. It will be worth investigating if other organisms with similar characteristics as *Deinococcus* can resist acute doses of irradiation.

4.3. Ecological Implications of ^{60}Co Gamma Irradiation on Soil Microbial Assemblage

Much attention is paid to ionizing radiation because it can prevent the preservation and spread of life outside the Earth as well as for environmental safety and industrial monitoring (Zhikrevetskaya et al., 2015; Cheptsov et al., 2018). From the results reported here, a valid generalized extrapolation can be made regarding the ecological effects of different doses of different radionuclides on soil microbial communities. For instance, that the ecological behaviour of radiocobalt is related to metal (iron and manganese) oxy-hydroxylases and their compounds (IRSN, 2010). Since these are essential for the myriad of processes within a radiation-resistant group of organisms as well as to enhance the immobility of the radionuclides. We can conclude that there is a dose-dependent toxicity effects of radiocobalt on soil microbial assemblage and the existence of a biomarker species from the phylum Deinococcus-Thermus. However, it will be useful to correlate the entire ecological effects with radiation response genes by conducting a high-coverage expression profiling (HiCEP). This tool (HiCEP) can uncover radiation-induced genes to record a response marker at the genomic level of a member of Deinococccus-Themus via dose-dependent gene expression in *Deinococcus ficus, D. deserti, D. geothermalis, D. grandis, D. murrayi, D. radiodurans,* etc.

The ecological (i.e. deterministic [non-stochastic] and probabilistic [stochastic]) effects observed in this study were controlled by the application of the radiation treatment in a weekly burst, which could have enabled a recolonization of the soil microbial community and creation of viable niches while reducing inherent effects. From this, we can conclude that ^{60}Co gamma irradiation is capable of reducing the diversity of soil microbial assemblage and possibly resulting in a proportionate reduction in their environmental services depending on the dose received. These characteristics of irradiated soil can be exploited in developing a homogeneous community of soil microorganisms as well as in the control of microbial proliferation.

Furthermore, the 16S rRNA and whole-genome shotgun metagenome sequencing revealed distinct community assemblage from the gamma irradiated soil except for the dominance of Deinococcus-Thermus. A more robust taxonomic assemblage was obtained from the 16S rRNA amplicon sequencing than from the whole genome shotgun metagenome sequencing. This is evidently due to the difference in sequencing approach. This is similar to what was reported in Tessler et al. (2017) and Delforno et al. (2017) when they used both approaches to characterize poultry slaughterhouse wastewater. Nonetheless, the functional profile of both approach revealed the same set of twenty-eight genes, which leads us to conclude that the integration of both approach allows for a greater coverage depth coverage of the unseen community that was irradiated as they augmented the shortfalls from each approach. Hence, the complimentary nature of both approach in radioecology was revealed in this study.

4.3.1. Deterministic outcomes in Soil Microbiome after ^{60}Co Gamma Irradiation

As a result of the irradiation, soil ecological systems experienced different biotic and abiotic stresses with varied communal response and expected specific individual response from known radiation-resistant organisms. These predictable effects can be used to prevent the establishment of response strategies as well as to prevent the potential long-term ecological consequences of irradiation on the soil. Continued radioecology research using this approach will contribute to establishing more deterministic effects on the microbial community from irradiation.

The applied doses and duration in this study led to the creation of 'radiation niches' in soil bacterial community dominated by *Hymenobacter* and *Streptomyces* in the low and medium treatments and *Deinococcus* in the high treatment respectively. There was also an activation of functional processes including DNA damage and repair response and alternative

energy pathways by microorganisms to help outlive the irradiation stress. Hence, we can conclude that these responses (functional and taxonomic establishment) are major determinants of dose, dose rate and radiation quality effects in cells.

The extended deterministic implication of this study subject the unpredictable outcome from soil irradiation, which ultimately depends on the soil type and nature of existing soil microbial community and the prevailing environmental conditions. Finally, the ecological safety of life below and above ground should be considered prior to handling and eventual disposal of radionuclides. The use of a radiation-contamination tracking system will help investigate source-sink dynamics of the microbiota as well as promote understanding of microbiome-exposure interactions on the ecology of the system if existing structure and function are to be maintained (Tasnim et al., 2017).

4.3.2. Stochastic effects on Soil Microbial Community of ^{60}Co Gamma Irradiation

Considering the central position of soil, the main **s**tochastic viewpoint is to prevent the entry of irradiation into the human food chain. Again, a dose-dependent stochastic relation is plausible since there were dose-dependent changes in the taxonomic assemblage and in the expression profile of stress-response genes in the present study. It is noteworthy that cases of dose-dependent irradiation expression changes are often characterized by high biological variability, displaying a stochastic nature of low dose radiation effects. Population and communal effects arise from effects on the individuals but the inverse is not always the case, because detectable effects in some members of a population would not necessarily have a consequence for the population as a whole (ICRP, 2008). Hence, the effects of 'umbrella species' on the community need to be further explored, since they may be central to the ecological consequences.

The danger of prolonged irradiation of soil microorganism is highlighted in the study. There is a risk of extinction of non-radiotolerant species and the evolution of a characteristic plastic radiation community that is different from those of the founding species. It will be fascinating to explore the stochastic effects of irradiation on the different stages of the soil microbial community. Whereby the severity is dose-independent but with a dose-dependent probability of occurring. As in other studies, this study cannot establish a threshold for the stochastic effects. However, this study has contributed towards the knowledge of deterministic outcomes after radionuclide-induced excitation of microbial cellular and subcellular structures including DNA damage, which will initiate other stochastic outcomes. Due to the nature of these stochastic factors, residual effects are likely to persist long after a new climax community is established after irradiation.

4.4. Future Directions and Policy Implications

This work has set a new pace in the field of radioecology by integrating community microbial ecology approach based on next-generation sequencing tools. This approach is capable of revolutionizing the field of radioecology by opening the gateway to hidden processes within soil communities subjected to ionizing irradiation. It can be expanded upon by applying other next-generation sequencing tools not adopted in this work due to limitations primarily cost e.g. transcriptomics and proteomics. Their application can give a more reliable insight into functional gene response. In addition, it can be used to mine and bio-prospect for useful metabolic biochemicals and radio-resistant organisms that can be harvested from an accidental spill or contaminated sites. With the advent of CRISPR techniques, transfer of genes from these radio-tolerant organisms can be used to create genetically modified organisms that can be used to bioremediate-contaminated sites. Whereas the harvested biochemicals can be used to produce industrial products in future. For instance, some members of Ascomycota can be targeted for their ability to capture and utilize energy from radionuclides.

Future studies are anticipated to adopt continuous irradiation of soil as opposed to the repeated burst adopted in the present studies. Furthermore, the collection of samples from actual contaminated sites in nature like Chernobyl and Fukushima or cities with a high application of gamma and other radiation sources like Daejeon and Ulsan cities in Korea Republic would be of great value. This will enable a greater application (in principle) of expected results. However, data obtained from these studies have provided the much-needed background on which future studies can be built upon. It will also be fascinating to exploit this approach to investigate other natural systems like the huge water resources around contaminated sites like Fukushima and elsewhere. In view of this aside from the metagenomics and metagenetic approaches reported here, transcriptomics and proteomics can also be

incorporated. Follow up studies may also incorporate a system enriched with known primary producers as this can help compare the effects of legacy carbon versus a biomass rich community and record the changes in functional processes.

The continued application of radionuclides for sterilization, wastewater and sludge treatment, preserving the integrity of artefacts and the potential of a nuclear war and accidents highlights the relevance of the approach reported here, hence it will be worthy to continue to investigate the impacts of radionuclides to natural assemblages of organisms. Some of the taxonomic and functional observation recorded from the irradiated soil showed similarities with soil from desert, hot springs and other extreme environments reported in other studies (Rainey et al., 2005; Connon et al., 2007; Wang et al., 2013; Cheptov et al., 2018), so it will be fascinating to incorporate all these in a single study. Due to the effects of soil type on the resulting effects of irradiated soil, it remains topical to delve into what amount and duration of a certain type of radionuclide are required to significantly alter a given soil community, which will doubtlessly be required to support policy implications (Tsubokura et al., 2016).

Moreover, the data presented here has contributed to filling the identified policy void regarding the impacts of radionuclides on soil systems. To this end, the immediate interpretation is to limit or avoid soil contamination by radionuclides. In the event of contamination, the soil should be immediately assessed and bio-remediated. Due to the mobility of radionuclides, above ground life forms like plants should be assessed to prevent possible transfer into other levels of the food chain. Nonetheless, it remains paramount to formulate and enforce a viable policy regarding soil contamination by radionuclides. This is in line with the IAEA's action plan on the protection of the environment (IAEA, 2005; ICPR, 2007)

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Appendices

*** Arranged in order of in-text mention**

Table A1. Chemical analysis of gamma radiation treated soil. pH value was close to neutral and did not differ much between and within treatment groups. Total organic carbon and total phosphorus varied between the treatment groups. Further, the percentage of total nitrogen did not show much difference.

Radiation level	pH	TOC (%)	TN (%)	TP (mg/kg)
Pre-treatment	7.90	1.81	0.159	506.18
Pre-treatment	8.00	1.85	0.162	511.91
Pre-treatment	7.90	1.69	0.148	519.52
Control	8.00	1.62	0.136	456.64
Control	8.00	1.58	0.142	427.00
Control	8.00	1.76	0.153	460.90
Low	8.00	1.51	0.137	481.34
Low	7.90	1.65	0.147	505.35
Low	8.00	1.57	0.145	490.58
Medium	8.00	1.65	0.143	493.15
Medium	8.00	1.60	0.108	474.23
Medium	8.10	1.53	0.137	452.92
High	8.00	1.63	0.074	507.43
High	8.00	1.65	0.146	501.19
High	8.00	1.60	0.047	505.10

Key:

TOC = Total organic carbon, TN = Total Nitrogen, and TP = Total phosphorus

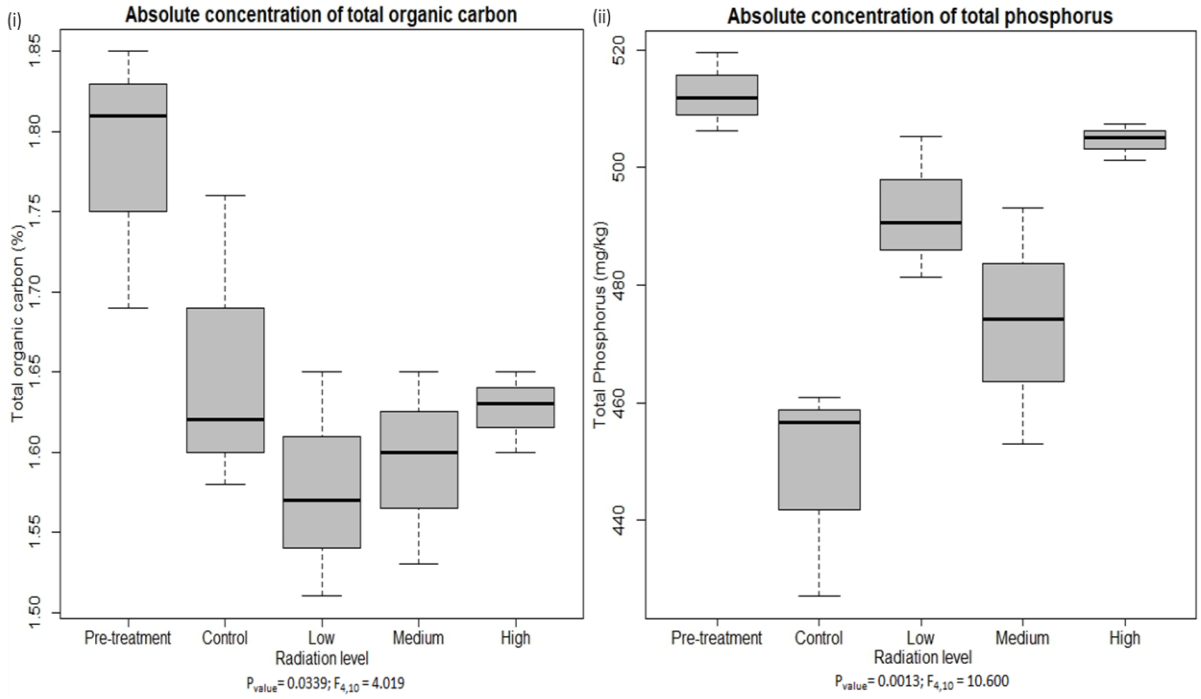

Figure A1. Concentration of (i) total organic carbon (%) and (ii) total phosphorus. Both were significantly affected by the radiation treatment and had P values of 0.0339 and 0.0013 respectively.

Table A2. Sampling site details and descriptive statistics of shotgun metagenomics sequences from MG-RAST

MG RAST ID	SAMPLE NAME	TREATMENT	TOTAL SEQUENCE	TOTAL BASEPAIRS	PREDICTED PROTEINS FEATURES	ANNOTATED PROTEIN (%)	TOTAL SEQUENCE AFTER QC	TOTAL BASEPAIRS AFTER QC
mgm4721502.3	0_C1	None	4,020,515	745,201,667	3,663,628	40.19	3,934,428	712,520,022
mgm4721506.3	0_C2	None	6,357,756	1,175,765,357	5,758,234	40.24	6,215,695	1,122,371,767
mgm4721490.3	0_C3	None	3,148,144	578,151,888	2,866,128	41.04	3,082,727	552,990,406
mgm4721496.3	0_L1	None	2,162,701	398,254,093	1,977,796	40.56	2,116,155	380,541,985
mgm4721494.3	0_L2	None	2,318,667	430,981,041	2,134,165	40.36	2,271,064	412,735,558
mgm4721497.3	0_L3	None	3,806,077	708,167,173	3,465,867	40.74	3,724,427	676,399,294
mgm4721492.3	0_M1	None	3,810,410	710,382,427	3,472,362	40.87	3,731,892	679,869,005
mgm4721488.3	0_M2	None	3,645,498	670,573,998	3,323,984	40.56	3,566,514	640,930,113
mgm4721498.3	0_M3	None	2,752,972	510,347,767	2,500,704	41.15	2,680,594	487,118,956
mgm4721486.3	0_H1	None	2,755,818	514,250,939	2,508,870	40.48	2,678,630	488,996,943
mgm4721504.3	0_H2	None	2,667,411	496,342,834	2,429,646	41.50	2,601,208	474,203,689
mgm4721487.3	0_H3	None	2,407,427	448,855,157	2,193,868	41.27	2,350,889	428,859,162
mgm4721505.3	6_C1	Incubated	3,155,188	589,106,649	2,872,552	42.30	3,082,270	561,024,270
mgm4721484.3	6_C2	Incubated	2,348,918	443,450,842	2,146,821	42.55	2,299,901	424,208,521
mgm4721500.3	6_C3	Incubated	2,498,942	458,426,991	2,271,650	42.10	2,442,713	436,928,719
mgm4721507.3	6_L1	Low	2,268,404	419,733,441	2,043,005	41.55	2,217,205	401,576,911
mgm4721485.3	6_L2	Low	2,410,004	444,400,399	2,171,479	41.61	2,352,304	424,580,126
mgm4721491.3	6_L3	Low	2,765,204	516,701,461	2,490,832	41.58	2,691,587	492,412,319
mgm4721503.3	6_M1	Medium	2,708,675	500,609,136	2,438,989	39.58	2,636,909	477,324,074
mgm4721495.3	6_M2	Medium	2,714,041	498,515,017	2,405,817	40.23	2,644,195	476,211,037
mgm4721489.3	6_M3	Medium	2,684,903	498,156,264	2,323,701	41.66	2,611,144	473,974,492
mgm4721493.3	6_H1	High	2,381,554	439,867,724	2,002,821	44.34	2,320,677	419,693,735
mgm4721499.3	6_H2	High	2,406,621	446,508,932	2,068,648	43.46	2,344,144	425,279,848
mgm4721501.3	6_H3	High	2,123,729	395,585,447	1,758,491	45.00	2,066,819	376,572,737

Key: Low (at 0.1 kGy), Medium (at (1 kGy) and High (at 3kGy) gamma ^{60}Co radiation treatment

(a)

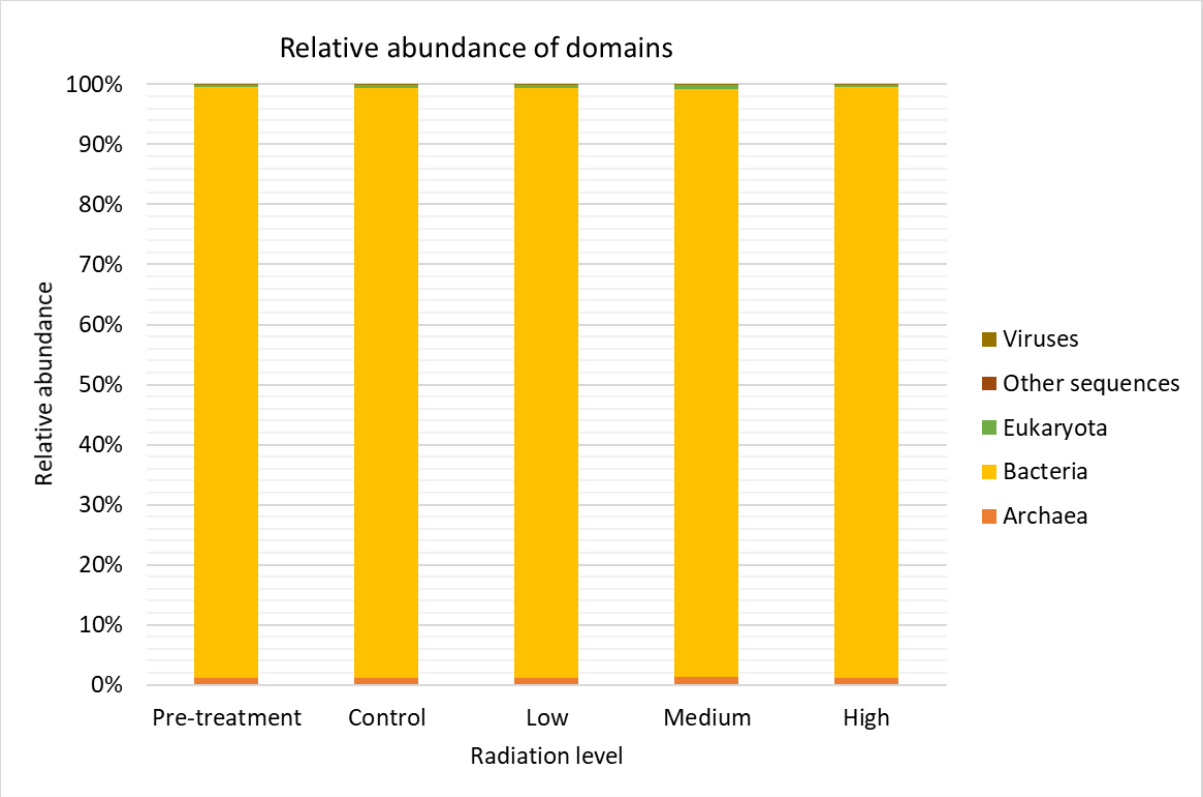

Figure A2a. Relative abundance of domains observed in shotgun metagenomic sequence data after exposure to different levels of gamma radiation. The most abundant domain is Bacteria followed by Archaea.

(b).

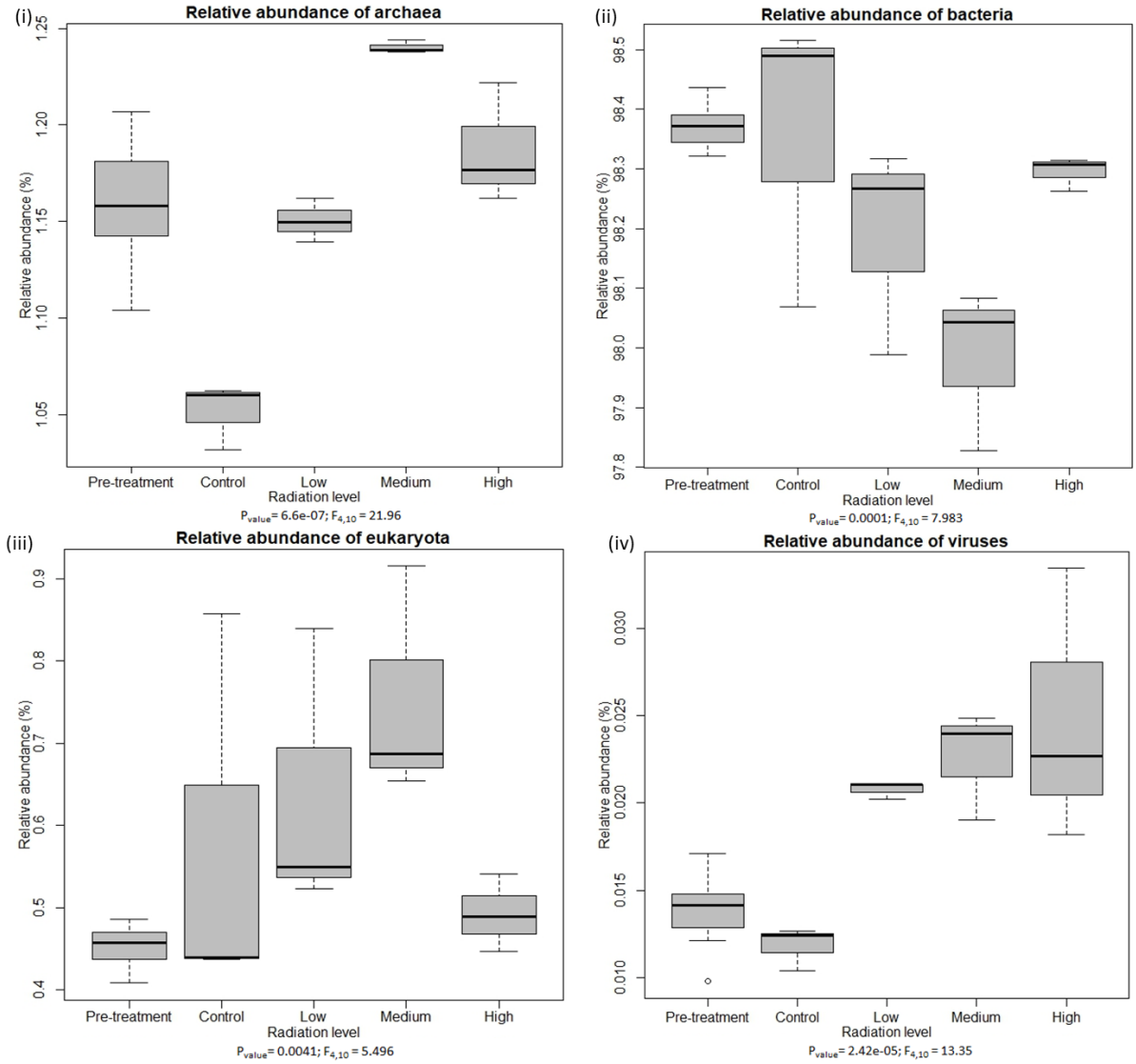

Figure A2b. The relative abundance of all domains were significantly different at $p \leq 0.05$. The relative abundance of Archaea increased while that of Bacteria decreased with increasing irradiation, whereas the relative abundance of Eukaryota and Viruses increased in low and medium treatment but reduced under high treatment doses.

(c).

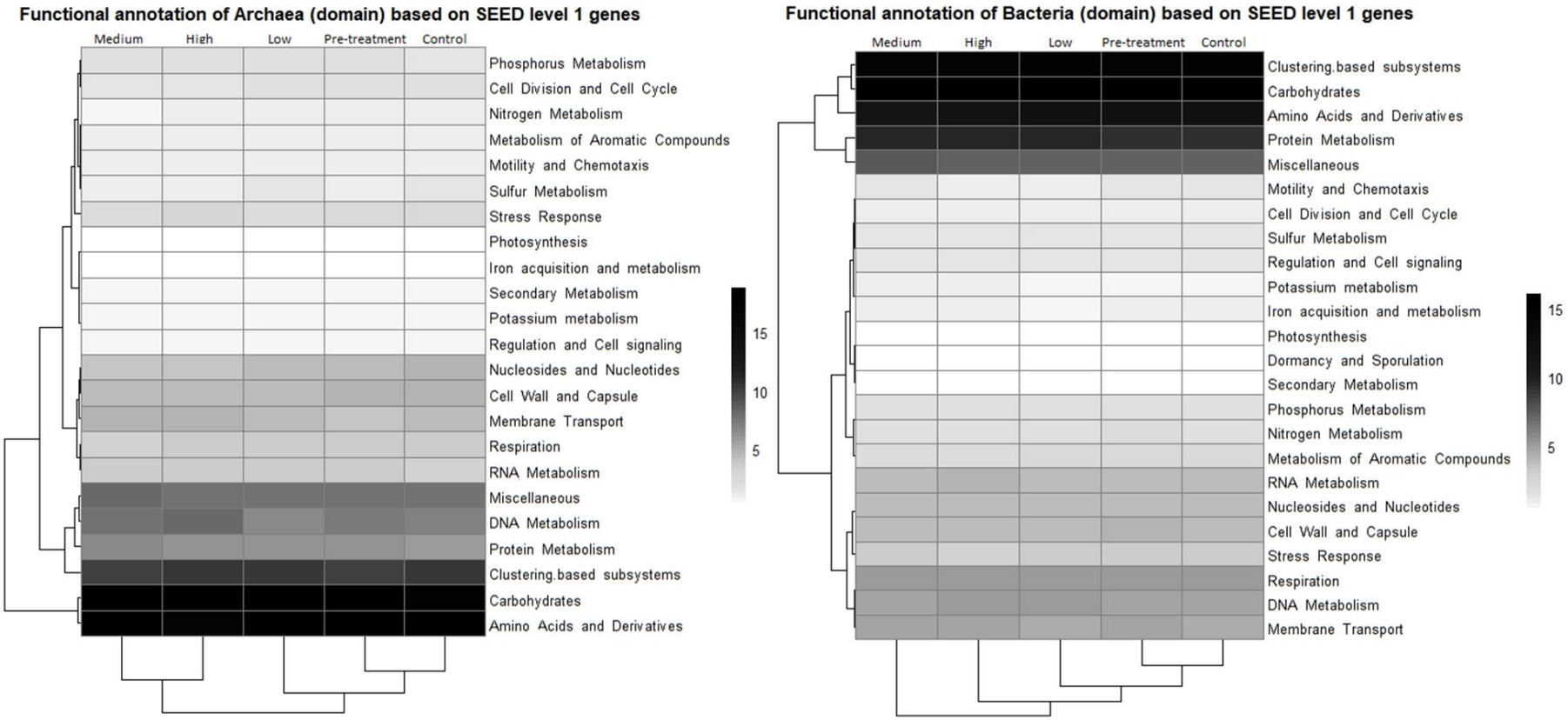

Figure A2c. The functional annotation of major domains. The annotation suggests that Archaea and Bacteria reveal different set of dominant genes may be responsible for their abundance. To varying degrees, Bacteria and Archaea were sustained by clustering based subsystems, carbohydrates as well as amino acids and derivatives related genes depending on the radiation intensity.

(d).

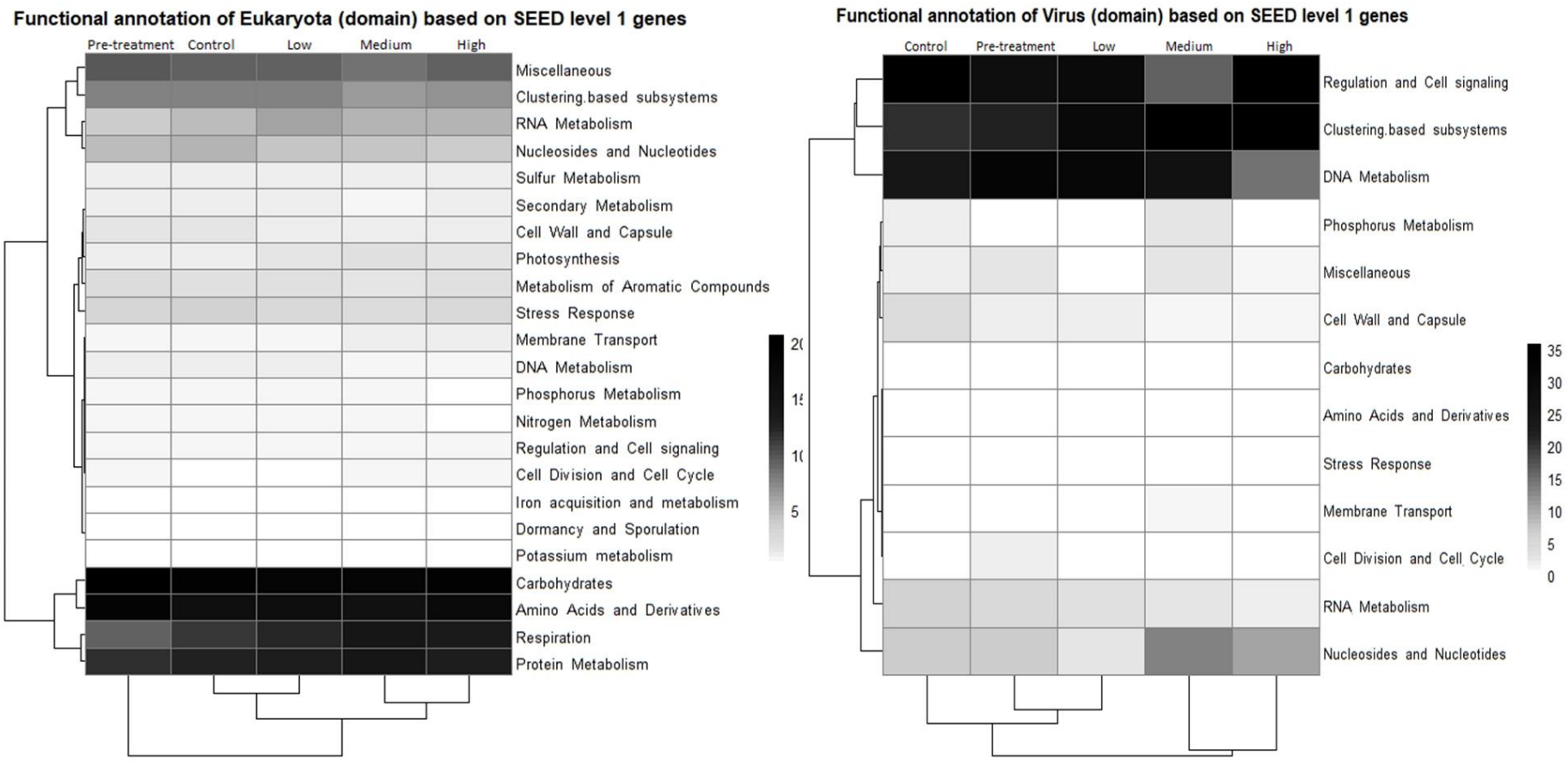

Figure A2d. The functional annotation of Eukaryota and Virus domains. The MG-RAST annotation suggests Carbohydrate and Amino acids were the major genes utilized by Eukaryotes while Viruses were sustained by clustering based subsystems, regulation and cell signalling as well as by DNA metabolism-related genes.

(a).

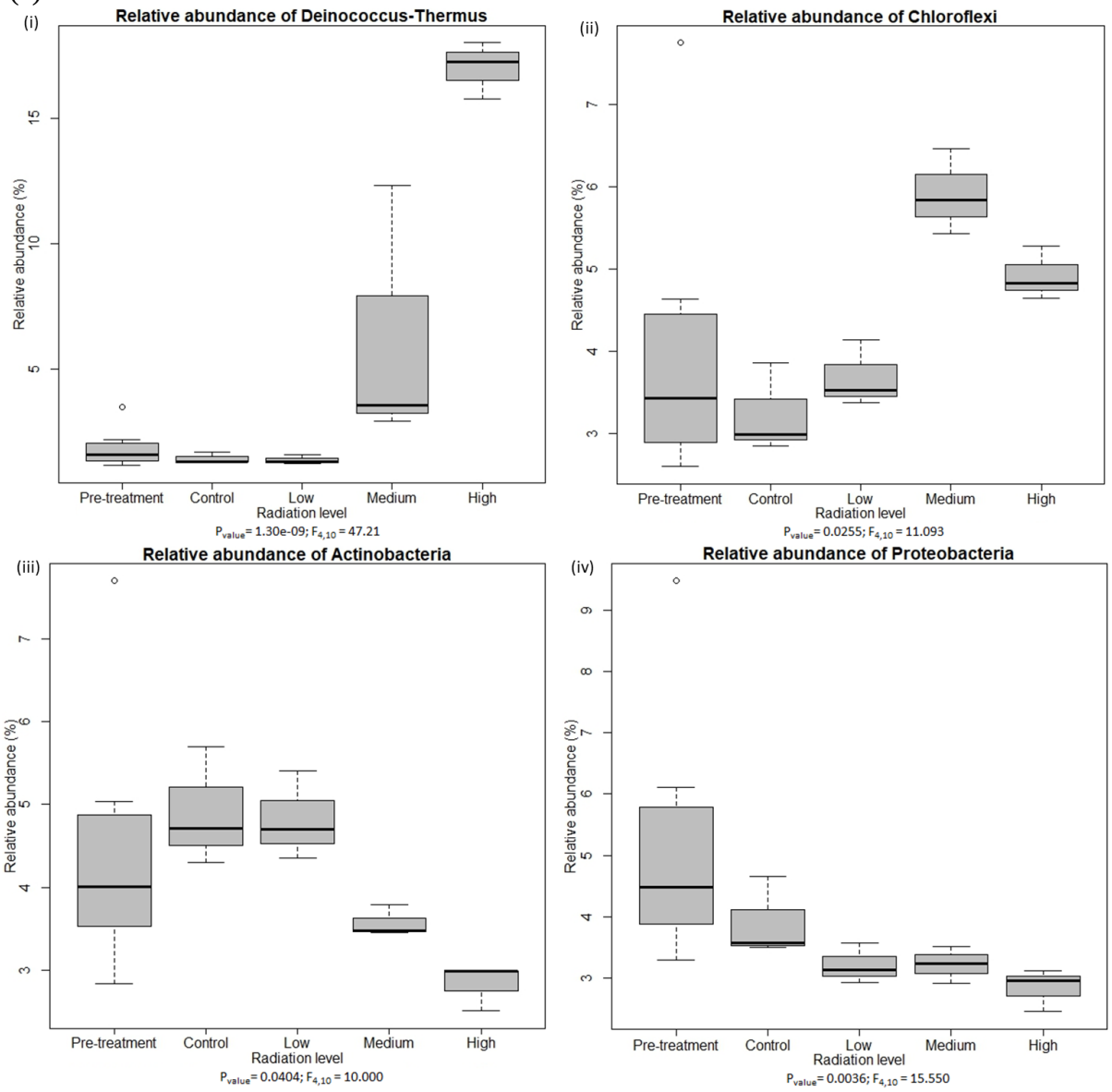

Figure A3a. The relative abundance of most abundant bacteria phyla across all the treatment levels.

(b).

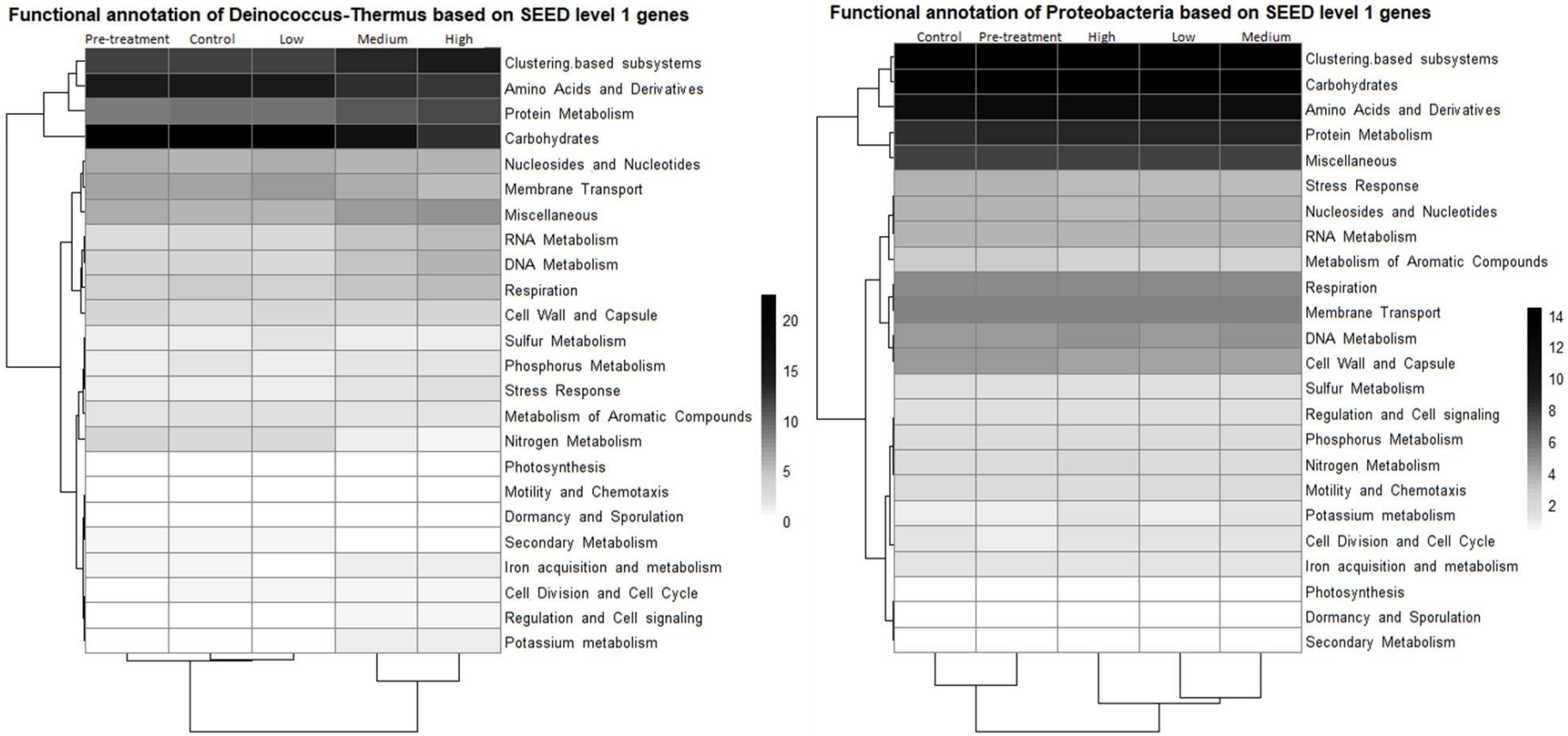

Figure A3b. The functional annotation of the most abundant bacteria phyla across all the treatment levels. The annotation reveals that although the diversity (in terms of numbers) of genes were similar the major group differ in abundance. Deinococcus-Thermus had greater annotation for carbohydrates while it was clustering based subsystems related genes in Proteobacteria (b).

(a).

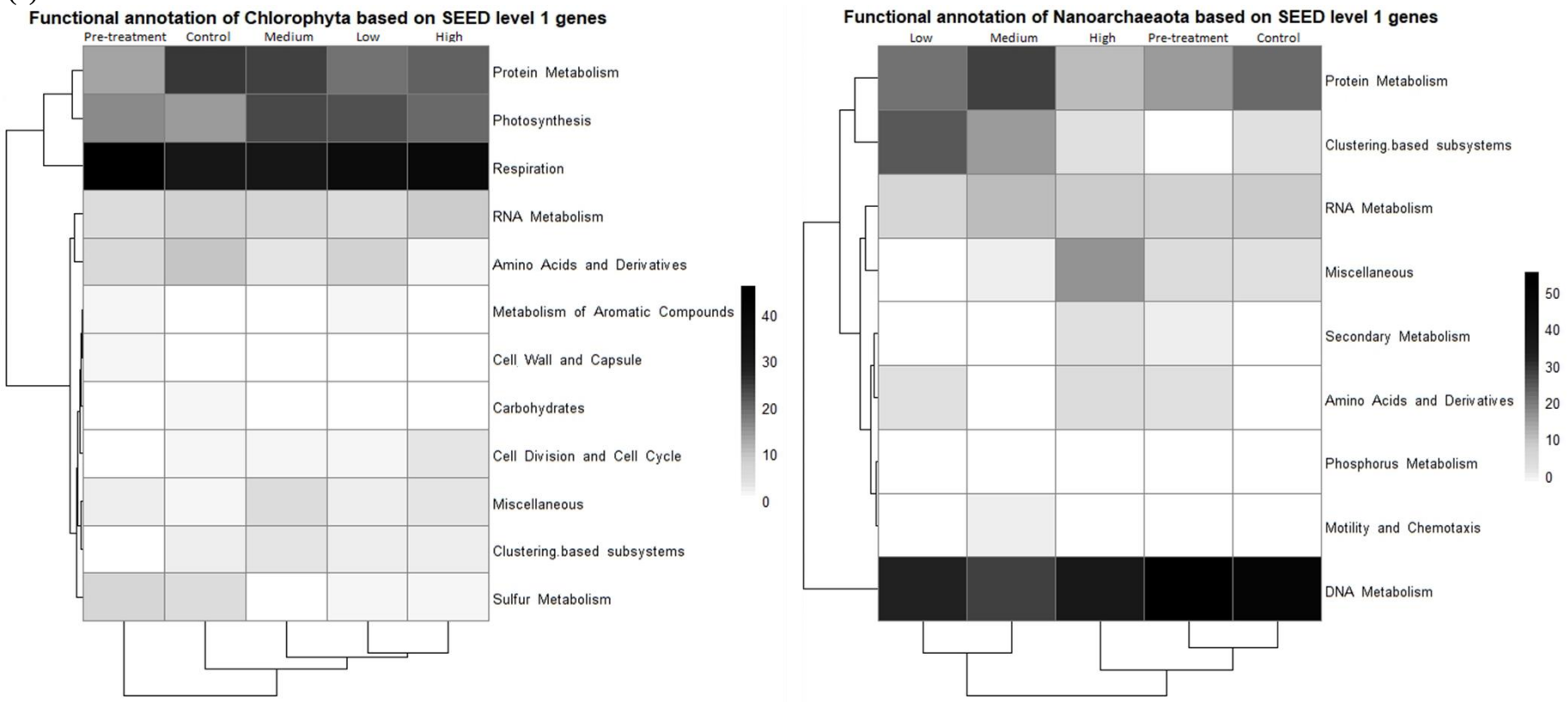

Figure A4a. Functional annotation of most abundant Eukaryota (Chlorophyta) and Archaea (Nanoarchaeota) (Figure A5a),

(b).

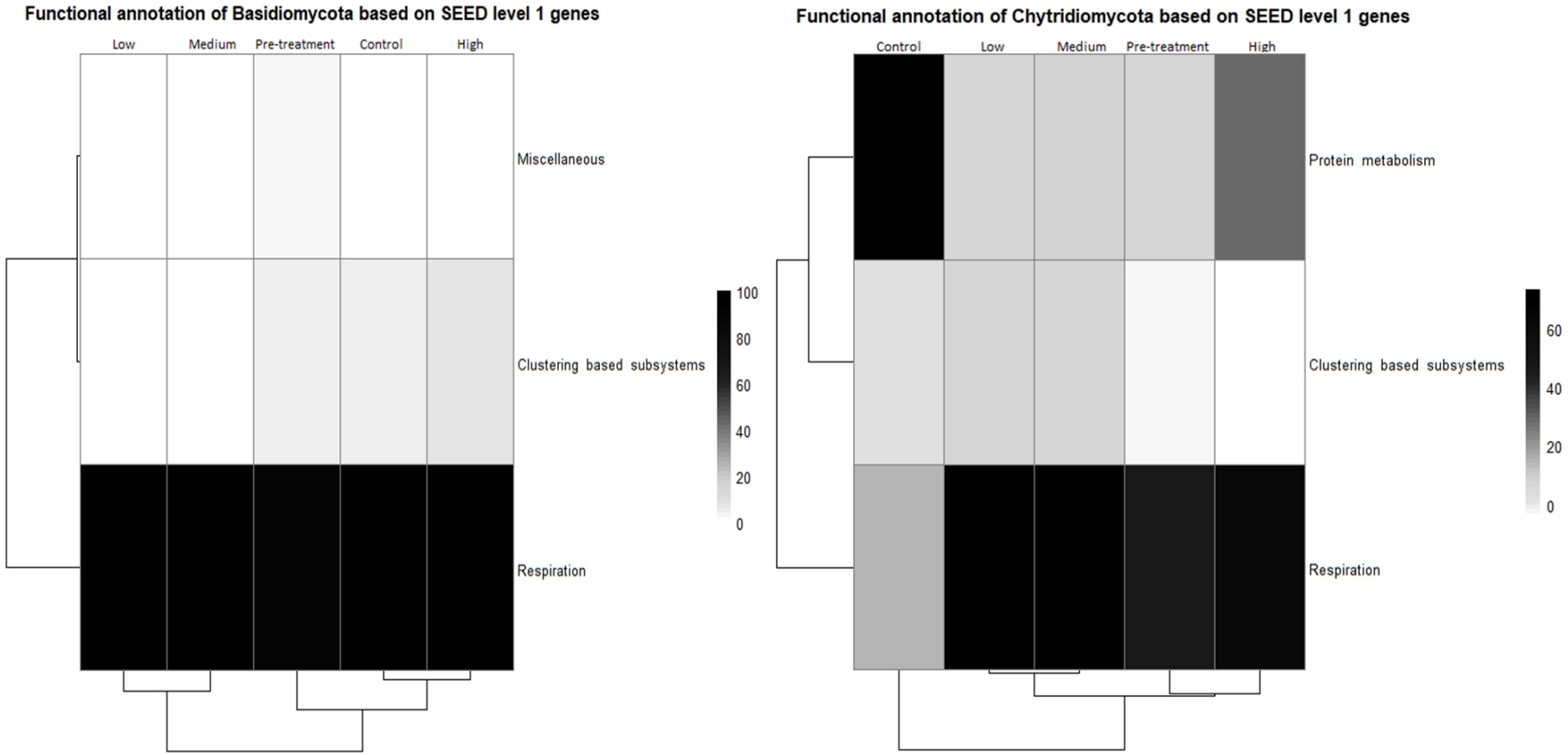

Figure A4b. Functional annotation of Fungi (Basidiomycota and Chytridiomycota).

Table A3. Shannon diversity index for the taxonomic group and SEED subsystems functional gene levels. Results showed all the different levels were statistically significant but for the domain (at the subsystem taxonomic level).

	Taxonomic level							SEED Functional gene levels			
	Domain	**Phylum**	**Order**	**Class**	**Family**	**Genus**	**Species**	**Strain**	**Level 1**	**Level 2**	**Level 3**	**Level 4**
X^2 or F value	14.313	19.173	16.889	16.875	19.02	19.553	19.153	19.153	16.107	7.053	18.463	17.328
P_{value}	0.0064	0.0007	0.0020	0.0020	0.0008	0.0006	0.0007	0.0007	0.0023	0.0011	2.43e-06	3.87e-06
DF	4	4	4	4	4	4	4	4,10	4	4,10	4,10	4,10
Control – Pre-treatment	+	+	*	*	+	*	*	*	*	+	+	+
High - Pre-treatment	+	*	+	+	*	*	*	*	+	+	*	*
Low - Pre-treatment	*	+	*	*	+	*	*	*	*	*	*	*
Medium - Pre-treatment	+	*	+	*	*	*	*	*	*	+	*	*
High – Control	+	*	*	*	*	*	*	*	*	+	*	*
Low – Control	+	+	+	+	+	+	+	+	*	+	*	+
Medium – Control	*	*	*	*	+	+	+	+	+	+	*	*
Low - High	+	*	*	*	*	*	*	*	*	*	*	*
Medium - High	*	+	+	+	*	*	*	*	+	*	+	+
Medium - Low	+	*	*	*	+	+	+	+	*	+	*	*

Key: + = Non-significant (at $P \leq 0.05$)

* = Significant (at $P \leq 0.05$)

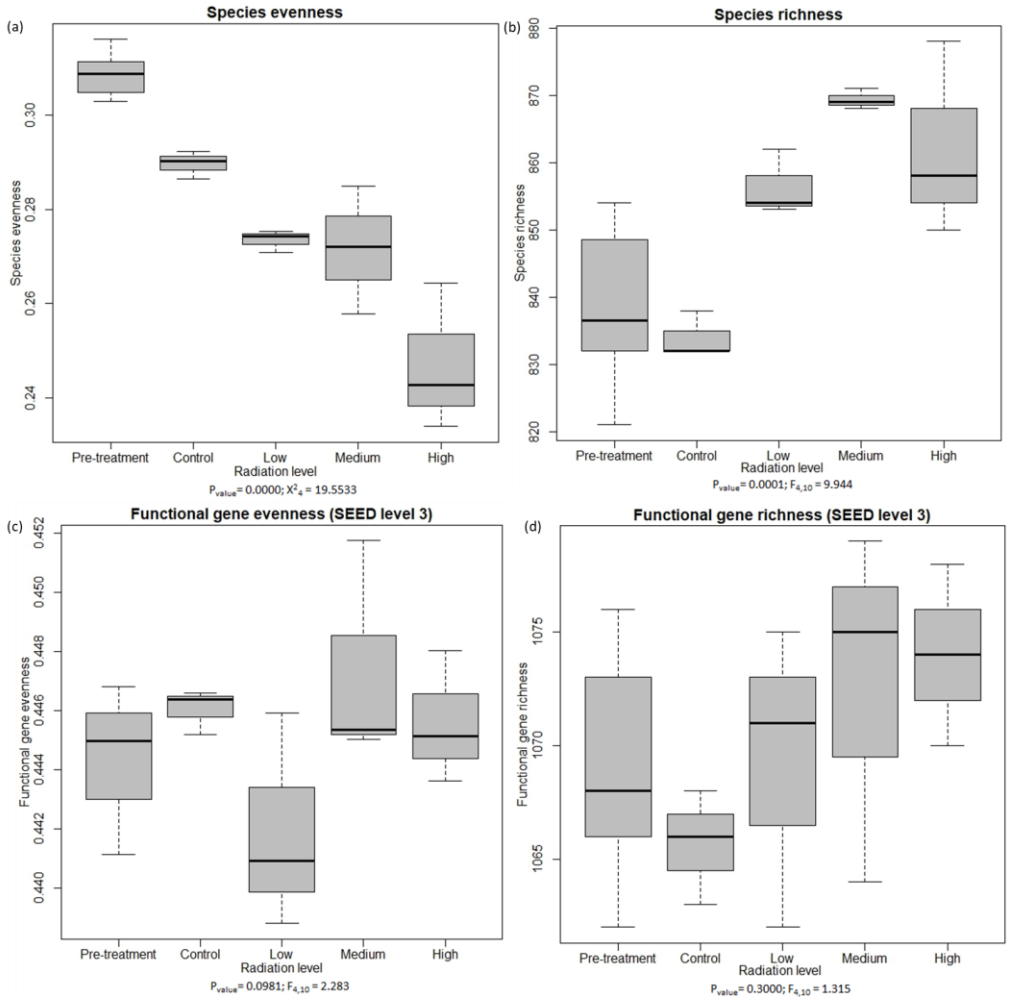

Figure A5. Species evenness and richness for taxonomic species and functional genes (SEED level 3). The species evenness and richness significantly reduced and increased with increasing radiation intensity respectively but the evenness of functional gene (SEED level 3) varied with treatment whereas the richness of functional genes (level 3) increased under irradiation.

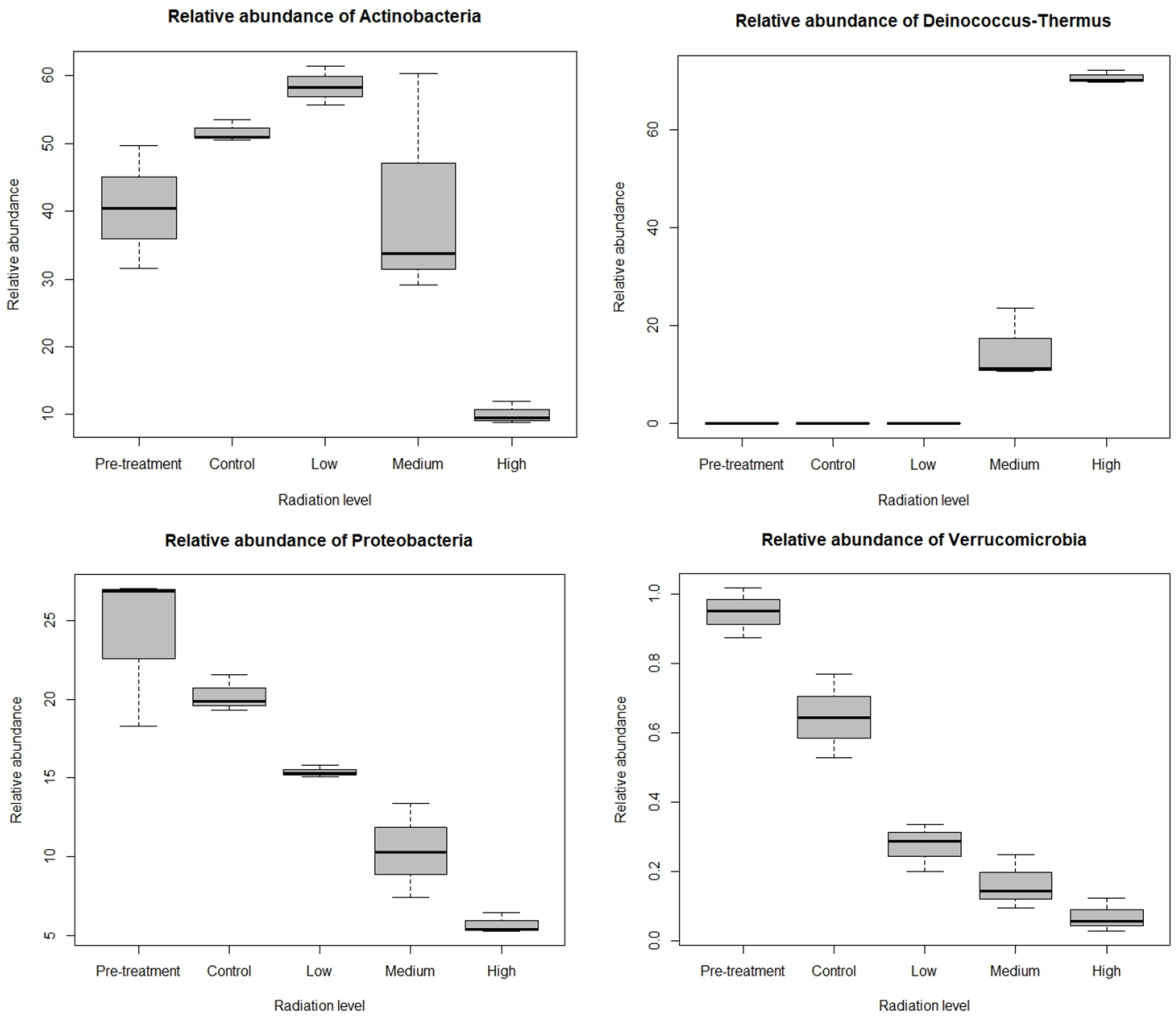

Figure A6. Relative abundance pattern of some bacteria phyla in the different treatment groups. Actinobacteria dominated the pre-treatment, control and low radiation treatment communities whereas Deinococcus-Thermus increasing dominated the higher radiation treatment communities. Proteobacteria and Verrucomicrobia decreased as gamma radiation treatment increased.

Table A4. Preliminary summary for the comparative genome analysis of the different *Deinococcus* species found on NCBI

Domain	Status	Genome Name	Total gene count	CDS genes (%)	No. of Pseudo Genes
Bacteria	Finished	*Deinococcus deserti* VCD115	3511	98.29	-
Bacteria	Permanent Draft	*Deinococcus ficus* DSM 19119	4075	98.16	-
Bacteria	Finished	*Deinococcus geothermalis* DSM 11300	3148	97.84	18
Bacteria	Draft	*Deinococcus grandis* ATCC 43672	4019	98.31	-
Bacteria	Permanent Draft	*Deinococcus murrayi* DSM 11303	2756	97.93	-
Bacteria	Finished	*Deinococcus radiodurans* ATCC BAA-816	3249	97.91	-

Key: CDS – Coding region sequence

Table A4 cont'd

Genome Name	Pseudo Genes (%)	Uncharacterized Genes (%)	No. of genes in KEGG	Genes in KEGG (%)
Deinococcus deserti VCD115	0.00%	0.00%	1009	28.74
Deinococcus ficus DSM 19119	0.00%	0.00%	984	24.15
Deinococcus geothermalis DSM 11300	0.57	0.00%	941	29.89
Deinococcus grandis ATCC 43672	0.00%	0.00%	1004	24.98
Deinococcus murrayi DSM 11303	0.00%	0.00%	804	29.17
Deinococcus radiodurans ATCC BAA-816	0.00%	0.00%	873	26.87

Table A4 cont'd

Genome Name	No. of transmembrane proteins predicted	Predicted CAZyme family genes						
		GHs	GTs	PL	CEs	AAs	CBM	Total
Deinococcus deserti VCD115	688	35	23	0	8	0	12	78
Deinococcus ficus DSM 19119	814	35	33	0	8	0	16	92
Deinococcus geothermalis DSM 11300	627	32	32	0	9	0	13	87
Deinococcus grandis ATCC 43672	777	36	25	0	8	0	15	84
Deinococcus murrayi DSM 11303	526	24	20	0	4	0	8	56
Deinococcus radiodurans ATCC BAA-816	616	21	22	0	7	0	11	61

Keys: GHs-Glycoside Hydrolases (GHs); GTs-GlycosylTransferases (GTs); PLs-Polysaccharide Lyases (PLs); CEs-Carbohydrate Esterases (CEs); AAs: Auxiliary Activities (AAs); CAZymes-Carbohydrate-active-enzymes

Table A4 cont'd

Genome Name	N. of genes in KEGG Orthology (KO)	No. of genes in COG	Genes in COG (percentage)	No. of TIGRfam clusters
Deinococcus deserti VCD115	1704	2285	65.08	838
Deinococcus ficus DSM 19119	1716	2434	59.73	859
Deinococcus geothermalis DSM 11300	1599	2060	65.44	883
Deinococcus grandis ATCC 43672	1746	2410	59.97	880
Deinococcus murrayi DSM 11303	1373	1779	64.55	798
Deinococcus radiodurans ATCC BAA-816	1566	2115	65.1	829

Table A4 cont'd

Genome Name	No. of CRISPR's	GC %	Total no. of bases	No. of genes in biosynthetic cluster	Genes in biosynthetic (%)
Deinococcus deserti VCD115		63	3855329	124	3.53
Deinococcus ficus DSM 19119	1	70	4146328	239	5.87
Deinococcus geothermalis DSM 11300	8	66	3247018	103	3.27
Deinococcus grandis ATCC 43672		70	4092497	208	5.18
Deinococcus murrayi DSM 11303	3	69	2767614	60	2.18
Deinococcus radiodurans ATCC BAA-816		67	3284156	109	3.35

Table A4 cont'd

Genome Name	Previous Isolation source	NCBI Bioproject ID	Short description
Deinococcus deserti VCD115	Gamma irradiated Sahara desert sand in Morocco and Tunisia	PRJNA16691	A Gram-negative, rod-shaped, and non-motile rods bacterial strain with cells present as pairs or in chains of four cells (de Groot et al., 2005)
Deinococcus ficus DSM 19119	Rhizosphere of *Ficus religiosa* in Taiwan	PRJNA188857	An aerobic, non-motile and Gram-staining-positive bacterial strain (Matrosova et al., 2017).
Deinococcus geothermalis DSM 11300	Uranium-resistant bacterium isolated from a hot spring	PRJNA13423	A thermophilic radiophile for bioremediation of radioactive mixed waste environments isolated from thermal springs at Agnano, Naples, Italy (Makarova et al. 2007).
Deinococcus grandis ATCC 43672	Radio resistant bacterium isolated from freshwater fish in Japan	PRJDB4362	A Gram-negative, red-pigmented, radioresistant, rod-shaped bacterium from freshwater in Japan (Satoh et al., 2016)
Deinococcus murrayi DSM 11303	Hot springs in Portugal	PRJNA188858	A Gram-positive, thermotolerant, catalase positive, non-motile, non-sporulating, sphere shaped bacterium
Deinococcus radiodurans ATCC BAA-816	Gamma irradiated ground pork and beef in Oregon, USA.	PRJNA315481	Can survive starvation, oxidative stress, and high amounts of DNA damage because they possess the capacity for DNA repair, DNA damage export, desiccation and starvation recovery, and genetic redundancy (White et al., 1999)

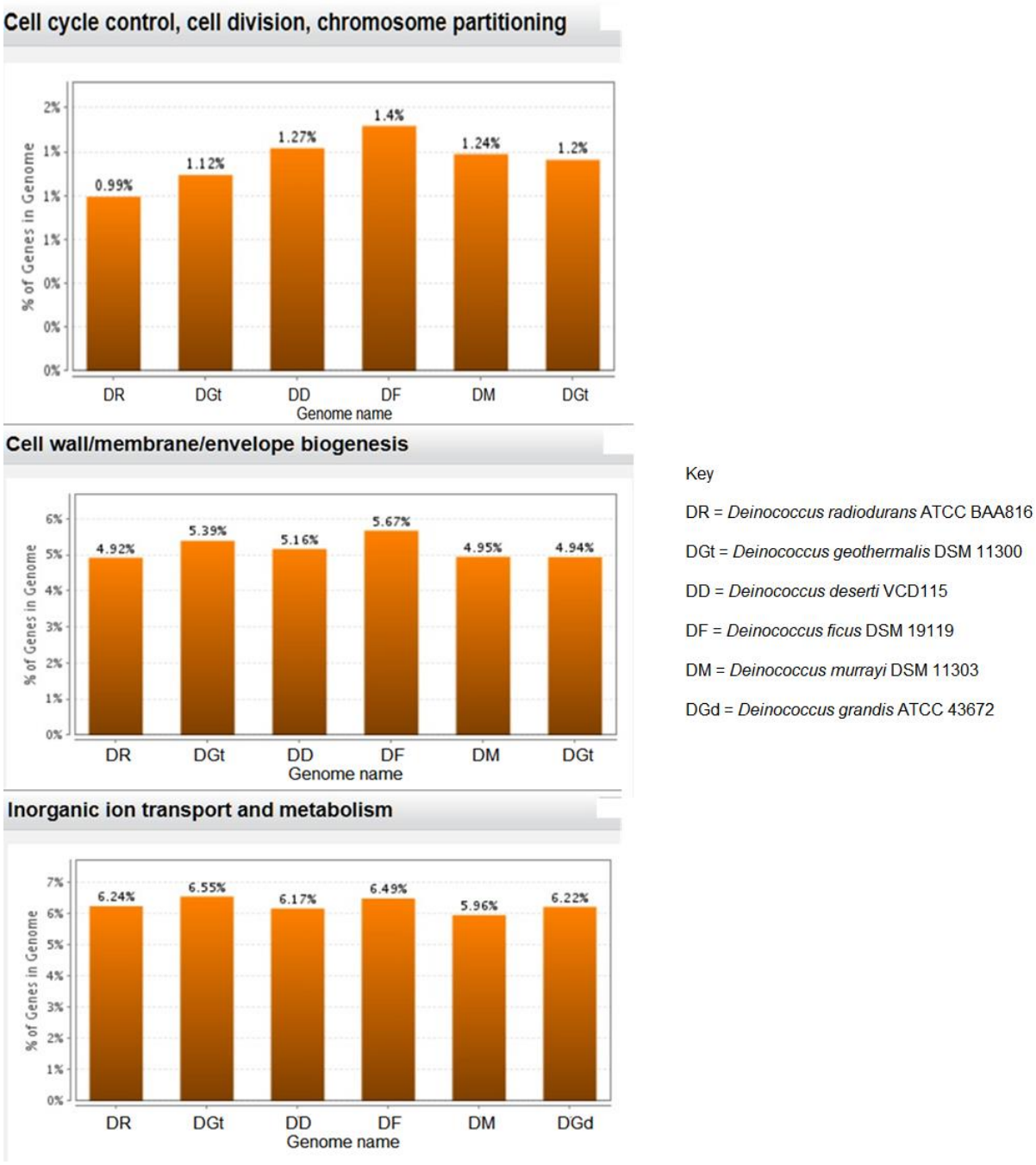

Figure A7. Comparative genome analysis from published genomes of the abundant *Deinococcus* species recorded in the study based cell cycle, cell wall and inorganic ion transport COG categories.

Table A5. RAST subsystems comparison of the different *Deinococcus* species.

Subsystem categories	***D. deserti* VCD115**	***D. ficus* DSM 19119**	***D. geothermalis* DSM 11300**	***D. grandis* ATCC 43672**	***D. murrayi* DSM 11303**	***D. radiodurans* ATCC BAA-816**
Cofactors, vitamins, prosthetic groups and pigments	104	110	98	104	80	99
Cell wall and capsule	28	12	25	28	12	22
Virulence, disease and defense	36	44	46	45	33	33
Potassium metabolism	4	8	6	8	5	9
Photosynthesis	0	0	0	0	0	0
Miscellenous	21	24	22	23	19	20
Phages, prophages, transposable elements and plasmids	0	0	1	0	3	1
Membrane transport	100	104	94	86	76	67
Iron acquisition and metabolism	5	3	0	0	3	0
RNA metabolism	42	40	39	36	32	39
Nucleosides and nucleotides	84	88	104	112	86	111
Protein metabolism	201	205	188	203	189	193
Cell division and cell cycle	4	4	5	29	4	4
Motility and chemotaxis	0	0	0	0	0	0
Regulation and cell signalling	11	9	11	11	13	13
Secondary metabolism	5	6	5	5	5	7
DNA metabolism	53	54	70	52	64	48
Fatty acids, lipids, and isoprenoids	66	78	66	71	69	73
Nitrogen metabolism	11	16	13	11	6	7
Dormancy and sporulation	2	1	1	2	1	1
Respiration	60	63	66	74	60	61
Stress response	41	30	25	37	16	24
Metabolism and aromatic compounds	32	17	29	15	13	13
Amino acids and derivatives	270	260	234	249	182	250
Sulfur metabolism	8	8	8	10	7	7
Phosphorus metabolism	29	34	29	33	27	34
Carbohydrates	178	152	163	170	140	157

Table A6. CRISPR protein counts in the differentially abundant *Deinococcus* species

CRISPR protein family	***Deinococcus deserti* VCD115**	***Deinococcus ficus* DSM 19119**	***Deinococcus geothermalis* DSM 11300**	***Deinococcus grandis* ATCC 43672**	***Deinococcus murrayi* DSM 11303**	***Deinococcus radiodurans* ATCC BAA-816**
CRISPR-associated protein, Csx2 family	0	0	1	0	1	0
CRISPR-associated protein, Cas1 family	0	0	2	0	3	0
CRISPR-associated protein, Cas2 family	0	0	2	0	2	0
CRISPR-associated helicase, Cas3 family	0	0	1	0	2	0
CRISPR-associated nuclease, Cas3 family	0	0	0	0	0	0
CRISPR-associated exonuclease, Cas4 family	0	0	1	0	1	0
CRISPR-associated protein, Cse1 family	0	0	1	0	1	0
CRISPR-associated protein, Cse3 family	0	0	1	0	0	0
CRISPR-associated protein, Cse4 family	0	0	1	0	1	0
CRISPR-associated protein, Cas5e family	0	0	1	0	1	0
CRISPR-associated protein, Csd1 family	0	0	1	0	0	0
CRISPR-associated protein, Csd2 family	0	0	1	0	1	0
CRISPR-associated protein, Csd5d family	0	0	1	0	0	0
CRISPR-associated protein, Cmr1 family	0	0	1	0	1	0
CRISPR-associated protein,Cmr2 family	0	0	1	0	1	0
CRISPR-associated protein, Cmr3 family	0	0	1	0	1	0
CRISPR-associated protein, Cmr4 family	0	0	1	0	1	0
CRISPR-associated protein, Cmr5 family	0	0	1	0	0	0
CRISPR-associated protein, Cmr6 family	0	0	1	0	0	0
CRISPR-associated protein, APE2256 family	0	0	1	0	1	0

Table A7. Count abundance of *Hymenobacter* species (Bacteroidetes). They are abundant in low and medium radiation doses, likely forming their own radiation niches under those treatment conditions.

Species	**PT1**	**PT2**	**PT3**	**C1**	**C2**	**C3**	**L1**	**L2**	**L3**	**M1**	**M2**	**M3**	**H1**	**H2**	**H3**
Hymenobacter actinosclerus	0	0	0	0	0	0	1	0	0	44	163	32	5	12	3
H. aerophilus	11	6	10	48	80	100	223	274	215	224	544	100	5	3	0
H. antarcticus	2	5	11	0	11	15	26	0	26	0	1	53	1	4	0
H. fastidiosus	3	3	7	8	12	7	12	16	19	233	439	217	3	8	1
H. ocellatus	10	8	172	19	41	27	21	34	22	316	618	263	13	23	7
H. roseosalivarius	0	0	2	0	0	0	0	2	0	158	346	164	0	8	8
H. sp. VUG-A141a	5	1	30	4	14	4	14	10	6	63	116	49	8	39	5

Keys: PT1 – Pre-treatment 1
PT2 - Pre-treatment 2
PT3 - Pre-treatment 3
C1 – Control 1
C2 - Control 2
L1 – Low 1
L2 – Low 2
L3 – Low 3
C3 - Control 3
M3 – Medium 3
H1 – High 1
H2 – High 2
H3 – High 3
M1 – Medium 1
M2 – Medium 2

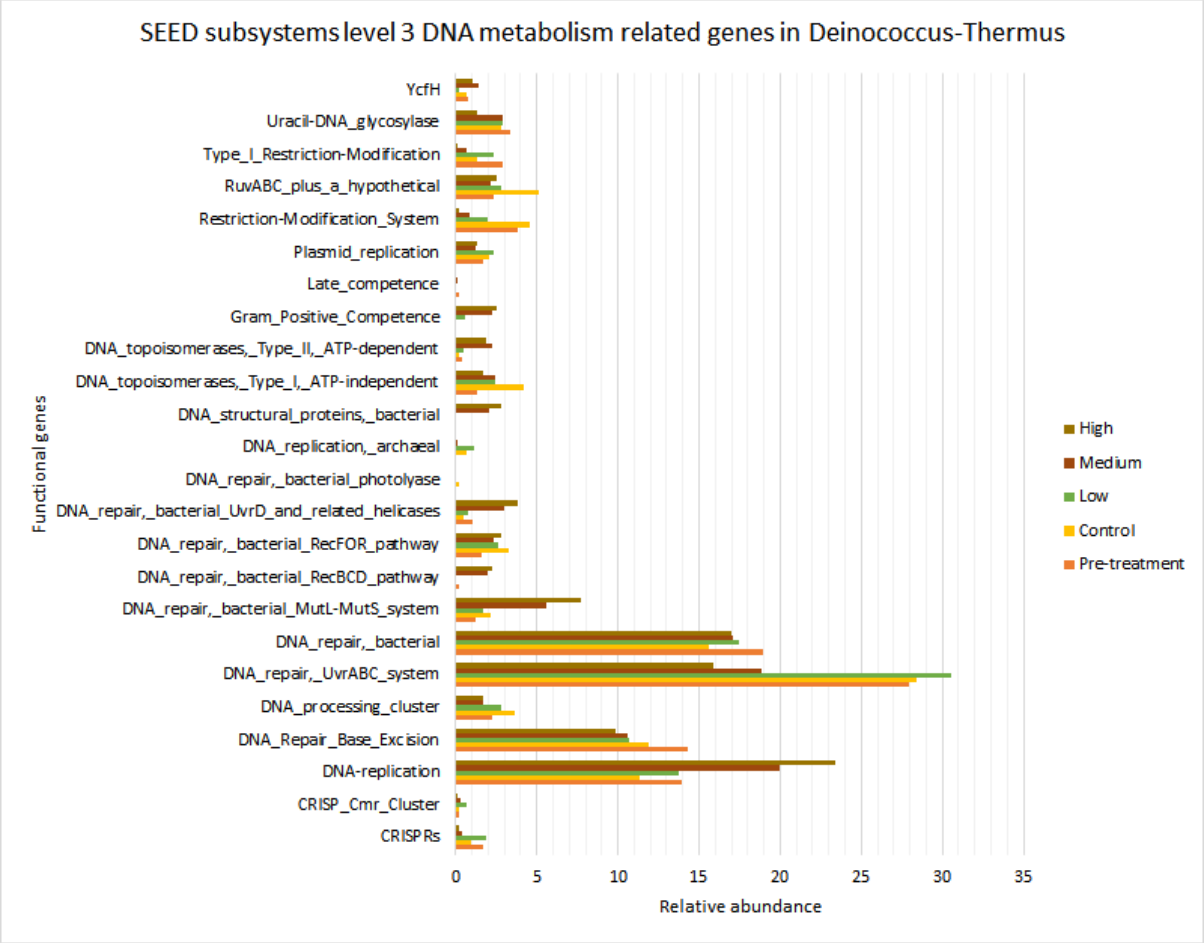

Figure A8. Annotation of SEED subsystem level 3 DNA metabolism-related genes for Deinococcus-Thermus. The result revealed higher DNA replication under high irradiation conditions.

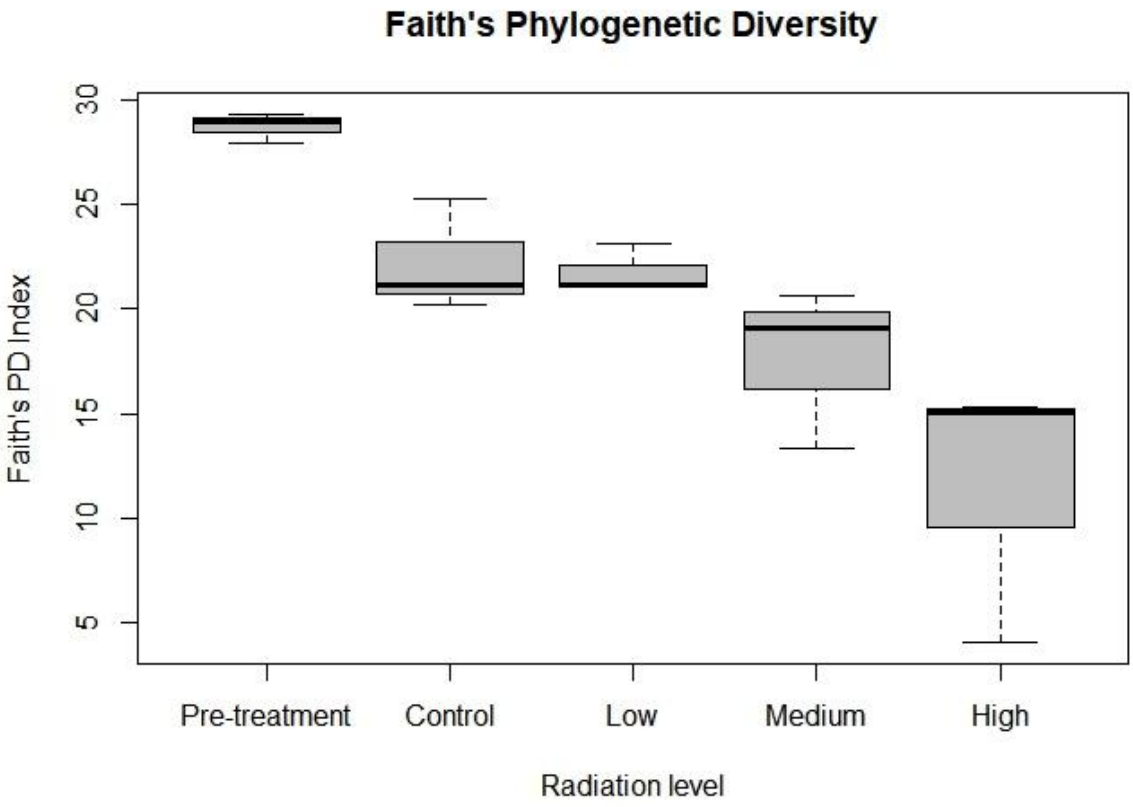

Figure A9. Phylogenetic diversity of the whole bacterial community. Phylogenetic diversity decreased with increasing radiation treatment.

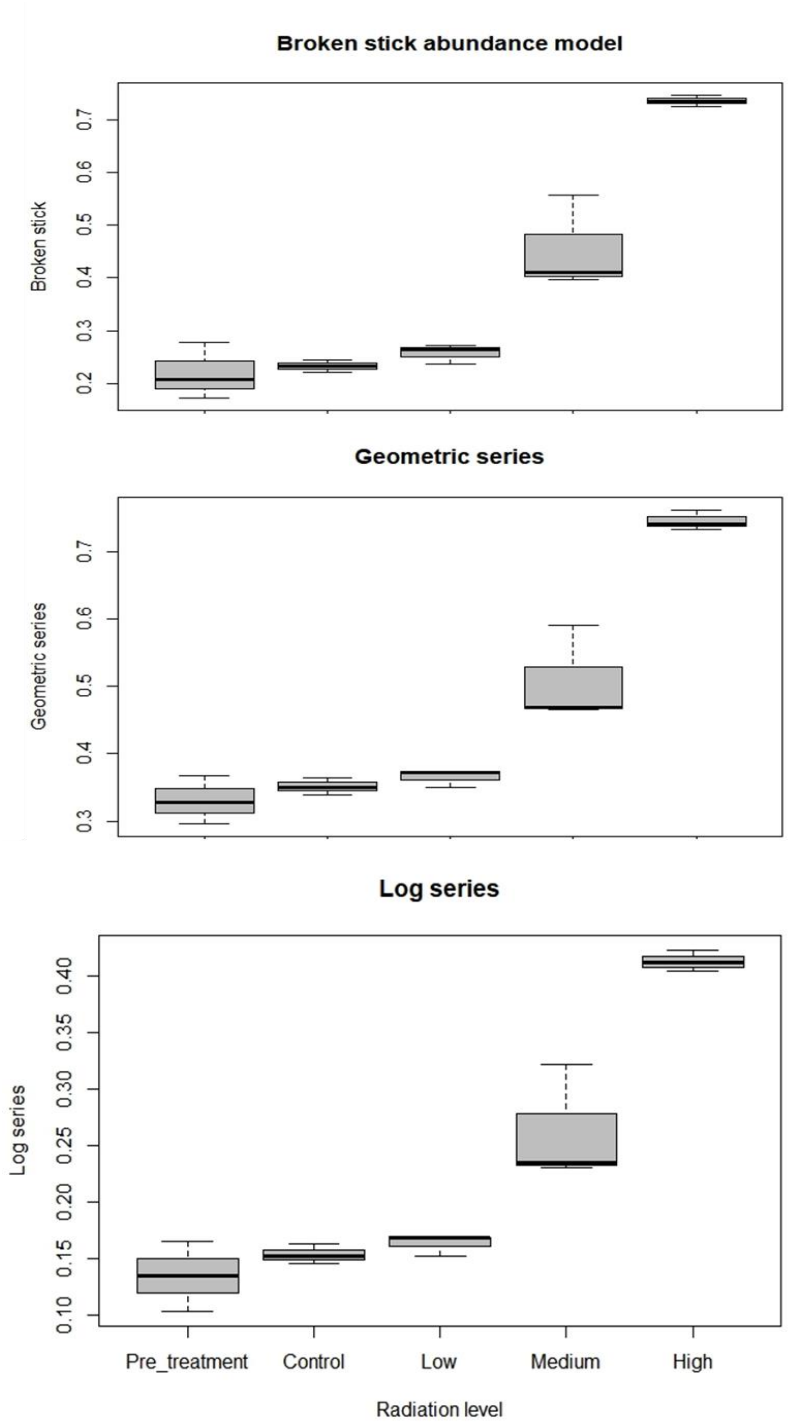

Figure A10. OTU abundance pattern within the different treatment levels. The three abundance models considered in the study showed a uniform increasing pattern with increase in ionizing radiation intensity. This suggests that the evenness and richness within each community increased due to gamma radiation.

국문초록 (Abstract in Korean)

이온화방사선이 환경에 초래하는 영향에 대해 알아보기 위해서는 생태학적 접근이 필요하다. 그러나, 이온화방사선이 토양 생물군집에 주는 영향에 대해서 알려진 바가 많지 않다. 본 연구에서는 6주간 주당 24시간 동안 3가지 다른 농도(0.1 kGy/hr [저], 1 kGy/hr [중] and 3 kGy/hr [고])에서 토양을 ^{60}Co 감마선에 노출시켰다. 첫 번째 연구에서는 토양 내 DNA를 추출한 후 샷건 메타지놈 시퀀싱 (shotgun metagenome sequencing)을 하고 MG-RAST를 이용하여 분석했다. 방사선 노출이 증가할수록 분류학적 다양성과 기능적 다양성 모두 감소할 것으로 예상했다. 박테리아의 분류학적 다양성은 감소한 방면, 예상외로 균 및 조류의 다양성은 증가했는데, 아마도 경쟁으로부터 벗어나게 된 것이 이유로 추정된다. 박테리아 및 전체 생물군의 분류학적 다양성은 감소했으나, 박테리아, 균 및 전체 생물군의 기능적 유전자 다양성은 증가했다. 이는 스트레스 혹은 교란이 다양성을 증가시키는 효과가 있다는 것에 대한 새로운 예시로, 방사선 노출이 토양 생물로 하여금 기능적으로 더 광범위한 전략을 취하게 한다는 것을 암시한다. 반복적인 밀도 의존적 군집 생장의 붕괴 및 확장이 복권과 같은 효과 (lottery effect)를 주어 공존을 증진시키는 것일 수 있다. 방사선은 전반적인 군집 구성에 큰 변화를 주었다.

본 연구에서는 방사선에 내성이 있는 새로운 미생물 군을 제시한다: 전체 군집의 20%를 차지하는 Deinococcus-Thermus뿐 아니라 Chloroflexi (박테리아), Basidiomycota와 Chytridiomycota(균), 그리고 Nanoarcheota (고세균) 또한 방사성에 내성이 있는 것으로 보인다. 방사성 노출로 인해 바이러스와 전이인자 (transposon)의 상대적 빈도는 증가했는데, 방사능 스트레스를 받은 세포들의 저항성이 떨어졌기 때문으로 추측된다. 예상외로, 열 충격 (heat shock), 해독작용, 산(acid) 스트레스 및 저온 스트레스 등의 '스트레스' 관련 유전자의 상대적 빈도는 방사능 농도가 가장 높을 때 줄어들었다. 그러나, 휴면 관련 유전자(퍼시스터 세포 (persister cells), 포자의 탈수, 생성 및 생장 등)의 다양성 및 DNA 수리 관련 유전자는 예상대로 증가했다.

두 번째 연구는 *Deinococcus* (감마선에 저항성이 있다고 알려진 박테리아의 한 속(genus))의 생태적 특성에 초점을 맞추어 진행되었다. 6주간 방사선에 노출된 토양 내에서 DNA를 추출하여 16S rRNA 유전자의 시퀀스 데이터를 확보했고, 메타지놈 (metagenome) 데이터 및 기존에 발표된 *Deinococcus*의 전체 유전자 데이터를 이용하여 다음 물음에 대한 해답을 얻고자 했다: 1) 방사선 노출이 증가함에 따라 박테리아의 군집 구조는 어떻게 변화할 것인가? 그리고 *Deinococcus*에 속하는 다양한 종들이 방사선 농도에 따라 서로 다르게 우점할 것인가? 즉, '방사선에 따른 생태적 지위 (radiation niches)'가 존재할 것인가? 2) 어떤 유전적 특징이 *Deinococcus*가 방사선이 높은

환경에서도 생존할 수 있게 하는가? 3) 방사선에 노출된 토양에서 *Deinococcus*는 어떤 영양학적 특징을 가지며, 이를 통해 방사선에 노출된 토양 내 생태적 과정들에 대해 알 수 있는 것은 무엇인가?

본 연구의 결과는 다음과 같다: 1) 방사선 노출 농도가 높을수록 *Deinococcus*의 상대적 빈도는 증가했으며, 가장 높은 농도에서는 상대적 빈도가 80%에 육박했다. 방사선 농도에 따라 우점하는 *Deinococcus* 종이 상이했는데, 이는 방사선에 따른 생태적 지위가 존재함을 나타낸다. 3 kGy/hr의 농도에선 *D. ficus*로 추정되는 단 하나의 조작분류단위 (OTU: operational taxonomic unit)가 우점했다. 2) 기존에 발표된 메타지놈 데이터에서도 *D. ficus*가 *Deinococcus*에 속하는 다른 종들보다 더 복잡한 유전적 구조를 가지며, DNA 및 뉴클레오티드(nucleotide) 대사, 세포벽, 세포막 신생, 세포 분열 조절, 세포 분열 및 염색체 분할 관련 유전자가 상대적으로 더 많았다고 보고되었다. 또한 GC 비율도 *Deinococcus*에 속하는 다른 종에 비해 높다. 이러한 특징들은 유전자의 안정성에 기여할 것으로 생각되고 명백히 경쟁도가 높다고 할 수 있는 방사선 노출이 심한 곳에서도 우점할 수 있는 근거를 제공한다. 3) 기존에 발표된 유전연구 데이터를 기반으로 할 때, *D. ficus* 등을 포함하는 *Deinococcus* 속이 방사선으로 인해 죽은 미생물 세포로부터 유래한 탄소공급원 (아라비노스 (arabinose), 젖당, 아세틸 글루코사민 (N-acetyl-D-glucosamine) 등 C5-C12를 포함하는 복합체)과 식물로부터 유래한 토양

유기물질들(셀룰로오스 (cellulose), 헤미셀룰로오스 (hemicelluloses) 등)을 이용할 수 있다는 것을 알 수 있었다. 4) 방사선 노출이 가장 심했던 토양에서도 토양시스템 내에서의 기본적인 기능과 관련한 유전자들 (리그닌 (lignin) 분해, 인 (P) 가용화, 질소 고정 등)이 비록 낮은 농도이지만 여전히 존재한다는 것을 메타지놈을 통해 알 수 있었다. 고농도로 방사선이 노출된 토양의 회복력 및 지속 가능성에 대한 연구, 다양한 토양 유형에서의 *Deinococcus*의 우점도 및 토양 내 기능 변화에 관한 연구도 후속연구 주제로 흥미로울 것으로 생각된다.

본 연구를 통해 감마선에 노출된 토양 생물군집의 기능적 유전자 및 분류학적 구성에 대해 알아볼 수 있었다. 본 연구는 토양 미생물 군집이 이온화감마선 (ionizing gamma irradiation)의 다양한 노출 농도에서 살아남을 수 있는 능력이 있다는 것을 보여주었다. 이는 감마선이 토양 또는 다른 중요 물질들의 멸균과정에 널리 이용되는 것에 대한 중요한 시각을 제공하고 오염된 환경에 대한 이해를 도우며, 우주여행에 대한 가능성 및 다른 행성에서의 지구생명체의 생존 가능성을 제시한다. 반복적인 주기로 토양을 감마선을 노출시킨 본 연구와는 달리 지속적으로 토양을 감마선에 노출시킨 연구, 실제 방사선으로 오염된 토양샘플을 대상으로 한 연구, 기타 다른 차세대시퀀싱 (NGS: next generation sequencing)기법들을 이용한 연구 등의 후속연구들이 등장할 것으로 기대된다. 이는 생물적 환경 정화 (bioremediation) 및 정책 형성에 기여하고

유용한 생물, 유전자, 및 생활성 (bioactive) 물질들에 대한 연구 및 개발에도 도움을 줄 것이다.

핵심어: ^{60}Co 감마선, Deinococcus-Thermus, 토양학적 불균형 (edaphic dysbiosis), 환경 변화, 레거시 효과(legacy effects), 미생물군집, 토양 오염, 방사선 저항, 방사선 생태학

학생 번호: 2016-32956

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